Chapter Number

Delivery Methods to Target RNAs in the Kidney

Csaba Révész and Péter Hamar
Semmelweis University, Budapest, Hungary

1. Introduction

Significant improvements have been made during the last 20 years in the therapy of renal diseases including the broadening of treatment options. Gene therapy is a potential modality for renal diseases for which we are yet unable to offer specific treatment. In spite of the revolutionary progress in the field of gene therapy, delivery to achieve safe clinical application remains one of the biggest challenges in biomedical research. In the present chapter we focus on nucleic acid (NA) therapy targeting messenger (mRNA) and micro (miRNA) RNA in the kidney.

2. RNA interference

RNA interference (RNAi), the sequence-specific post-transcriptional gene silencing mediated by small (19-25 nucleotide length) double-stranded RNAs (dsRNA), is of potential use as a therapeutic approach for the treatment of a variety of diseases (Dykxhoorn & Lieberman, 2006; Castanotto & Rossi, 2009). Small, non-coding RNA molecules such as microRNA (miRNA) and short interfering RNA (siRNA) are important regulators of gene expression, helping to control cellular metabolism, growth and differentiation, to maintain genome integrity, and to combat viruses and mobile genetic elements (C. Zhang, 2009; Moazed, 2009).

2.1 Biogenesis of small RNAs and mechanism of action

Following completion of the human genome project, a series of non-coding small RNAs have been discovered. Two main categories of small RNAs have been defined on the basis of their precursors. The cleavage of exogenous double-stranded RNA (dsRNA) precursors produced during viral infection or after artificial transfection generates siRNAs, whereas the processing of genome-encoded endogenous stem–loop RNA structures generates miRNAs.
Exogenous siRNAs and endogenous miRNAs are generated from dsRNA precursors that are produced in or introduced into cells (Siomi H. & Siomi M.C., 2009; Bartel, 2004).
During the mechanism of RNAi, double-stranded RNA is cleaved by an RNAseIII ribonuclease called Dicer into smaller fragments (21 to 23 nucleotides). The resulting fragments are then bound to an Argonaute family protein which functions as the core component of a protein–RNA complex called the RNA-induced silencing complex (RISC) or its nuclear form the RNA-induced transcriptional silencing complex (RITS). SiRNA/miRNA duplex is unwind by helicase, and the guide or antisense strand will then engage in selective degradation of the mRNA that is complementary to the guide strand.

As a post-transcriptional gene-silencing (PTGS) mechanism **exogenous siRNA** mediated RNAi causes degradation of the target mRNA and prevents protein synthesis (Rácz & Hamar, 2008). Physiologic function of RNAi is elimination of viral infections, as double stranded RNAs are often produced during the life cycle of viruses and are eliminated this way. However, siRNA mediated PTGS can be utilized therapeutically, by transfecting cells in vivo with siRNA complementary to a segment of a protein coding mRNA (Rácz & Hamar, 2006). The revolutionary aspect of siRNA therapy is that it acts on disease-associated key proteins by down regulating components of the pathway without permanent effects on the genome. Thus, siRNA treatment has the potential to prevent injury from occurring in addition to addressing existing injury.

Endogenous non-coding miRNAs are involved in post-transcriptional regulation of gene-expression. MiRNAs are generated from endogenous hairpin structured transcripts throughout the genome. MiRNA encoding genes are transcribed by RNA polymerase II (pol II) providing long precursor transcripts, known as primary miRNAs (pri-miRNAs). After transcription, Drosha RNase: a type III nuclear ribonuclease cleaves nucleotides from the pri-miRNA, processing it into shorter pre-miRNAs. Nuclear export factor Exportin-5 (Exp5/Xpo5) transports the pre-miRNA to the cytoplasm. Further cytoplasmic processing by Dicer (type III ribonuclease) cleaves pre-miRNA generating mature, double-stranded, 18-25 nucleotide-long miRNA. The guide strand is incorporated into RISC and remains stably associated with RISC, becoming the mature miRNA. The opposite (passenger) strand is disposed. The miRNA guides the RISC to the target mRNA with complementary sequence. In case of incompletely complementary sequences, translation of the target mRNA is silenced, and the mRNA is degraded by the RISC in case of fully complementary sequence. Unlike RNAi induced by siRNA, miRNA during regulation, cleavage of the mRNA occurs more seldom, only by complete match between the miRNA and the mRNA (Kaucsár et al., 2010).

MiRNAs and siRNAs control post-transcriptional gene expression by directing endonuclease cleavage of the target mRNA. This mechanism of action is referred to as slicer activity. For slicer activity fully complementary miRNA and target mRNA sequences are required. However, in some cases, few mismatches can be tolerated for cleavage. MiRNAs may also act a slicer-independent manner such as mRNA repression, or mRNA decapping (Valencia-Sanchez et al., 2006).

Regulatory miRNAs compose networks. This notion is supported by (i) the high number of non-coding RNAs which are functionally active and target signaling molecules, (ii) many genes encoding miRNAs are closely clustered in the genome and (iii) in some cases different miRNAs control a single mRNA target or vice versa a single miRNA may influence expression of multiple different target proteins. Human and murine kidney specific miRNA expression profiles have been reported and summerised (in Kaucsár et al., 2010).

2.1 Further small RNA types

A non-coding RNA (ncRNA) is a functional RNA molecule that is not translated into a protein. Non-coding RNAs include long known transfer RNA (tRNA) and ribosomal RNA (rRNA) involved in translation, as well as newly described RNAs involved in gene expression regulation such as :

- Small nuclear RNA (snRNA) - involved in mRNA splicing.
- Small nucleolar RNA (snoRNA) - direct the modification of ribosomal RNAs.

- Micro RNA (miRNA) and short interfering RNA (siRNA) - regulate gene expression.
- The P-element induced wimpy testis in Drosophila (piwi) (Saito et al., 2006) proteins regulate stem- and germ-line cell division (Cox et al., 2000). Piwi-interacting RNAs (piRNAs) isolated from mouse testes (Kim, 2006) are involved in defense of germ-line cells against parasitic DNA elements such as retrotransposons.

MiRNAs and siRNAs are about 20-25 nucleotides long. piRNAs have a broader average size ~24–31 nucleotides. siRNAs have been widely used in functional genomics and also have therapeutic potential.

3. Challenges in RNA-based therapy knocking down disease

Presently, therapeutic RNAi application face three major obstacles:
i. *stability* of siRNA when administered in vivo,
ii. *delivery* across barriers in living organisms, and
iii. circumvention of *immune response*.
Efforts to improve the effect of RNAi-based nucleic acid (NA) therapy include,
i. *stabilization* of the nucleic acid against enzymatic degradation,
ii. enhancing *cellular uptake* of the nucleic acid, and
iii. limiting *immunoactivation* during in vivo application.
Stability of siRNA in vivo in biological milieu is one of the obstacles for therapeutic application. Numerous modifications are available, since chemically synthetized siRNAs became cheap and easy to synthetize. (For chemical modifications see 4.2.).
Delivery problems include rapid degradation of NAs and rapid renal clearance. Double stranded NAs have an advantageous resistant profile against nuclease degradation which can be further reduced by chemical modifications. Rapid renal clearance eliminates the therapeutically applied small NA from the circulation, however, this can be utilized as an advantage in targeting the kidney as tubular epithelial cells (TEC) may take up the NAs from the ultrafiltrate. Thus, TEC can be efficiently targeted this way (Molitoris et al., 2009).
Exogenously administered siRNAs may have undesired side effects (Rácz & Hamar, 2008). Such unwanted side-effects may include: unintended knockdown of partially complementary sequences may occur resulting in off-target silencing. Side effects induced by siRNAs have been shown to be concentration dependent. Off-target silencing is more likely to occur in case of high siRNA concentrations but sometimes may occur even in low siRNA concentration (Jackson et al, 2003). Moreover, high siRNA concentration can also induce gene activation of apoptosis and stress response (Semizarov et al., 2003) or can lead to non-selective translational shutdown (erre nem találtam hiv-t).
Immunological response is an other obstacle in therapeutic siRNA application. Toll-like receptors (TLRs) recognize pathogen-associated molecular patterns (PAMPs), such as bacterial wall endotoxins (LPS), viral dsRNA, and cytosine-guanine (CG) motifs. TLRs also recognize siRNA, and consequently TLR intracellular signaling pathways are activated leading to immune activation. Moreover, dsRNAs induce interferon response directly.
Delivery across barriers in living organisms, e.g. across biological membranes is a major issue for potential therapeutic application of siRNAs. Chemical modifications, complexation, conjugation with lipid bilayer-penetrating carriers assist this purpose. This chapter focuses on delivery issues of RNAi and possible therapeutic influencing of miRNA expression targeting the kidney.

4. Delivery strategies

Delivery strategies to induce cellular uptake of the therapeutic nucleic acid include physical force or vector systems such as viral-, lipid- or complex- based delivery, or nanocarriers. From the initial applications with less possible clinical relevance, when NAs were addressed to renal cells with hydrodynamic high pressure injection systemically, a wide range of gene therapeutic viral and non-viral carriers have been applied already to target posttranscriptional events in different animal kidney disease models in vivo.

4.1 Physical approaches

One of the strategies for introducing NAs into cells and tissues is physical force. During these approaches hydrodynamic pressure is applied to force the NAs into tissue parenchyma and cells. The hydrodynamic pressure can be established systemically during the hydrodynamic procedure or locally during direct injections into the target organ.
Intrarenal, local delivery to the kidney can be achieved by different routes such as via the renal artery targeting glomeruli, via the renal vein targeting the tubulointerstitium, via the ureter into the renal pelvis and by subcapsular administration for intraparenchymal effects. Effects of the hydrodynamic pressure can be further enhanced by temporary pore openings in cell membranes by electroporation or sonoporation with ultrasound or micro-injection.
DY547-labeled non-target control siRNA or rhodamine-labeled p85α siRNA uptake followed by hydrodynamic or standard tail vein injection was noted in the kidney with consequent protein inhibition in case of p85α siRNA, but no signal was observed after intraperitonal or per rectum administration (Larson et al., 2007). Hydrodynamic administration resulted in a higher uptake in the kidney as well as in other target organs in contrast to standard intravenous injection (Larson et al., 2007).
In a mouse model of renal ischemia-reperfusion, Fas siRNA pretreatment hydrodynamically through the tail vein and/or injected locally into renal vein protected NMRI mice from renal ischemia-reperfusion injury (Hamar et al., 2004). Sufficient downregulation of the FAS apoptosis receptor substantially reduced functional deterioration of the kidney manifesting in significant survival advantage. Similar good results were achieved by silencing apoptosis cascade elements (Zheng et al., 2008) or the central inflammatory signaling element: nuclear factor kappa-b (NFkB) (Feng et al., 2009).

Apoptosis antagonizing transcription factor (AATF), a regulator of apoptotic pathways, was targeted in an *in vitro* model of kidney ischemia-reperfusion injury . In human kidney proximal tubule HK-2 cells and in primary renal tubule epithelial cells RNA interference–mediated silencing of AATF heightened whereas overexpression of transgenic AATF ameliorated superoxide accumulation and apoptotic cell death following hypoxia., (Xie & Guo, 2006).
Gremlin siRNA plasmid suspended in 2 ml of the TransIT-EE Hydrodynamic buffer delivered weekly by hydrodynamic tail vein injection reversed diabetic nephropathy in streptozotocin-induced diabetic, uninephrectomized mice. Furthermore, high glucose induced collagen IV and MMP-2 activity was inhibited by lipofectamin transfection of the same gremlin construct in cultured mouse mesangial cells (Q. Zhang et al., 2010). Gremlin is highly expressed in kidney with diabetic nephropathy, mainly observed in areas of tubulointerstitial fibrosis, and its mRNA level correlates with the degree of the fibrosis.

4.2 Chemical modifications

Spontaneous uptake of siRNA by cells without additional carrier is reportedly less efficient when compared to strategies employing transfection reagents that complex or encapsulate siRNA. *Chemical modification* and bioconjugation with vehicles can drastically improve the stability and cellular uptake, allowing improvements in selectivity and reduced toxicity. While phosphodiester oligonucleotides are unstable in the biological milieu, several chemical modifications have been applied to enhance stability.

Before RNAi was discovered antisense oligonucleotides were used for experimental nucleic acid sequence specific inhibition of protein synthesis. Main differences between siRNA used in RNAi and previously popular antisense oligonucleotides (ASOs) is, that ASOs are single stranded and thus, much more sensitive to extracellular nuclease degradation, whereas siRNAs are more resistant due to their double stranded structure (Paroo & Corey, 2004).

First generation of chemically modified oligonucleotides was phosphorothioate RNA, later 2′ sugar modifications (2′-O-methyl and 2′-O-methoxy-ethyl-RNAs) were introduced (Monia, 1997). The hydrophilic character and anionic backbone of siRNAs reduces their uptake by the cells. While siRNA duplexes are relatively more stable in serum than single stranded siRNA, they easily undergo degradation by nucleases in vivo. This, with their limited capability to cross cellular lipid bilayers, represents a significant barrier to the therapeutic development of siRNA. A variety of chemical modifications have been tested to enhance effectiveness of oligonucleotide intracellular delivery, including chemical modifications to sugars (2′-sugar), backbones, or nucleobases. Conjugation to membrane penetrating vehicles have been also demonstrated to enhance stability, prevent triggering of an immune response, control pharmacokinetic profiles and reduce nonspecific effects. However, some of these modifications may affect biological activity (Rácz & Hamar, 2006, 2008).

4.3 Viral vectors

Besides chemical modifications *vector systems* have been used widely to protect and deliver NAs in vitro and in vivo. Gold standards of gene delivery are *viral vectors*. Several kinds of viral vectors have been used already in delivery applications into the kidney. Strategies employed in previous gene therapy applications can be readily adapted for use in RNAi. Viral vector containing expression cassettes coding for shRNA precursors, as an alternative to siRNA administration has been successfully used in the kidney employing plasmid DNA (pDNA) vectors. Few studies describe renal shRNA delivery with viral vectors such as, replication-deficient *adenoviral* delivery in rat cortical tubules, glomeruli or tubular epithelium of the outer medulla or *lentiviral* retrograde ureteral infusion to tubular epithelial cells in mice. Intraparenchymal delivery of lentivirus particularly induced transgene expression in the cortical and corticomedullary area of the kidney with lower expression in the medullary part. In a rat renal transplantation model, perfusion of the donor kidney with lentiviral vector induced significant target gene silencing. Transplantation offers an ex-vivo window enabling significant reduction of possible systemic side-effects of NA therapy. From a safety perspective, the use of lentiviral vectors may lead to unwanted insertion of the construct in vital gene regions.

Recombinant *adenoviruses and adeno-associated viruses (AVs, AAVs)* are capable of transducing cells with high efficiency. However, adenoviral vectors have been reported to be immunogenic. In mice, intraparenchymal or intrapelvic delivery of recombinant AAV induced transgene expression by epithelium of the tubules or mainly in the medulla,

respectively. Despite recent concerns, recombinant AAVs are attractive vectors as they appear to be safe and capable of long-term gene expression. Although efficient delivery vehicles, adenoviruses are strong stimulators of innate and adaptive immune responses. This may cause toxicity and limit repeated administration. To modify tropism and reduce immune responses, recent studies have used surface modified or helper-dependent 'gutless' vectors. Helper dependent and chemically modified vectors may have an improved safety profile that could be better suited to clinical application.
Adeno-associated (AAV) virus-2 vector was used for inhibiting mineralocorticoid receptor (MR) by MR-shRNA expressing AAV. AAV MR-shRNA reduced MR expression in the kidney and prevented blood pressure increase, albuminuria, and renal failure in cold-induced hypertension in Sprague–Dawley rats (Wang et al., 2006)..
Anti-luciferase siRNA-expressing piGENE hU6-stem21 pDNA was co-injected hydrodynamically with pGL3 firefly luciferase-expressing pDNA via tail vein in ddY mice in a dose- and time-dependence study of vector-based in vivo RNAi (Kobayashi et al., 2004). Authors investigated silencing efficiency in liver, kidney, lung and muscle. Viral vector mediated RNA silencing of the transgene 1 day after intravenous injection was almost as efficient in the kidney as in the liver.
Hemagglutination virus of Japan–envelope vector was used in uninephrectomized streptozotocin-induced diabetic mice to target mammalian translocase of inner mitochondrial membrane 44 (TIM44) (Y. Zhang et al., 2006). TIM44, a membrane anchor of mitochondrial heat-shock protein 70 (mtHsp70) to TIM23 complex is upregulated in diabetic mouse kidneys and is held responsible for superoxide production. RNAi to TIM44 reduced proteinuria, renal hypertrophy, renal cell proliferation and apoptosis, and suppressed superoxide production.

4.4 Non-viral carriers

Applying *non-viral* vehicles constitute promising alternatives to the use of viral vectors. Delivery of siRNA, especially with cationic preparations and biodegradable components have much better safety profiles than their viral counterparts, though their transfection efficiency is generally lower. The positive charge facilitates complex formation with NAs and endocytosis of the complex. The complex between the cationic carrier (lipids, polymers, peptides, nanoparticles) and the anionic NA is formed by electrostatic interaction.

4.4.1 Lipid-based delivery

Various lipid-based delivery systems have been developed for in vivo application of siRNA, including liposomes, micelles, emulsions, and solid lipid nanoparticles. Application of liposome-mediated gene carriers retrospects a long path from the seventies. Complexes between cationic lipid and DNA are named lipoplexes. Due to the high complexity of the self-assembly process, little is known about the mechanisms of formation. Multicomponent lipoplexes, incorporating three to six lipid species, have emerged as promising delivery candidates, with 10 to 100 times higher efficiency than binary complexes usually applied for gene delivery (Caracciolo et al., 2005, 2009). Our understanding about the bio-distribution of RNA-liposome complex is rather scarce, however, it is known to depend on the colloidal properties of the complex as well as their interaction with blood components. Surface charge is a main issue in half-life and destination of the complex. While lipoplexes with a strong anionic charge are usually absorbed by scavenger cells, resulting in a rapid elimination from

the blood, a strongly positive surface favors the accumulation in the liver (Y.-C. Tseng et al, 2009).

Liposome-based transfection reagents have been successfully employed for *in vivo* siRNA delivery to the kidney, such as

- cationic lipids
- N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulfate (DOTAP)
- (3β-[N-(N′,N′-dimethylaminoethane)-carbamoyl])-cholesterol (DC-Chol)

or neutral helper lipids

- 1,2-dioleoyl-*sn*-glycero-3-phosphatid-ylcholine (DOPC),
- dioleoylphosphatidylethanolamine (DOPE).

Protection of nucleic acids with encapsulation into the liposome makes these lipid delivery strategies attractive for gene transfer. However, composition and surface charge pattern are important issues in organ targeting and potential immune recognition and response.

Lipoproteins have also been tested as lipid-based transfection reagents. Efficient and selective siRNA conjugation to bile acids and long-chain fatty acids, or cholesterol, depends on interactions with lipoprotein particles, lipoprotein receptors and transmembrane proteins (Wolfrum et al., 2007). High-density lipoprotein (HDL) directs siRNA delivery into the liver, gut, kidney and steroidogenic organs, whereas low-density lipoprotein (LDL) targets siRNA primarily to the liver. In a delivery study of lipoprotein-siRNA complexes it has been ascertained that ^{32}P-cholesterol-siRNA bound to HDL or albumin but not to LDL resulted in an accumulation in the kidney, while with LDL the main target organ was the liver.

Examples of successful lipid based siRNA delivery in renal pathologies

Intravenous RNAi-mediated inhibition of p53 delivered by lipofectamine transfection agent minimized renal injury in a hypoperfusion/ischemia and a cisplatin model of kidney damage in rats. P53 siRNA minimized renal p53 protein increase after ischemia-reperfusion injury. Proximal tubule cells were protected against ischemic and cisplatin-induced acute injury by chemically modified, 2′O-methylated p53 siRNA (Molitoris et al., 2009). Following i.v. injection, rapid glomerular filtration with subsequent proximal tubule brush border binding and endocytosis by proximal tubule cells were observed. The minimal labeling in the vasculature indicated rapid renal clearance.

Renal injury and diabetic nephropathy were ameliorated by cholesterol-tagged 12/15-lipoxygenase (12/15-LO) targeting siRNAs in streptozotocin-induced mouse model of type 1 diabetes (Yuan et al., 2008). Lipoxygenases (LO) are a family of non-heme iron-containing enzymes that insert molecular oxygen into polyunsaturated fatty acids. The 12/15-LO is involved in mesangial cell growth and extracellular matrix protein expression during glomerulosclerosis. Cholesterol conjugation was applied to enhance renal siRNA uptake. Significant reduction of 12/15-LO mRNA level was observed in murine mesangial cells in vitro, and the examined tissues in vivo (spleen, kidney, liver, and heart), indicating broad tissue biodistribution of the siRNAs. Glomerular hypertrophy and mesangial matrix expansion were reduced.

Breast adenocarcinoma (MDA-MB-231) cells in vitro and in female SCID mice in vivo were treated with cationic liposome loaded with COX-2 siRNA (Mikhaylova et al., 2009). COX-2, is often upregulated in cancer. DOTAP/DOPE (DD) and DOTAP/DOPE/DOPE-PEG2000 (DDP) lipid mixtures were used for producing lipoplexes. For imaging purposes liposomes were loaded with different contrast agents. Incubation of DDP lipoplexes for extended periods demonstrated siRNA-dependent, sequence-specific downregulation of COX-2

protein expression in breast cancer cells. Also, tumor-bearing female SCID mice treated with DDP-COX-2 lipoplexes DY-647 labeled localization of COX-2 siRNA was observed in the tumor, lung and liver shortly after injection. In contrast, the fluorescence intensity in the kidney was lower, however, second highest signal of detectable fluorescence was observed in the kidneys 24h postinjection.

Physical approaches (such as hydrodynamic in vivo delivery) are often combined with adaptation of different carriers. DY547- and rhodamine-labeled, chemically modified siRNAs mixed with DOTAP effectively appeared in the kidney after hydrodynamic and standard iv. injection, while i.p. and rectal administration were unsuccessful even with DOTAP liposomal transfection reagent (Larson et al., 2007).

RLIP76 multifunctional transporter, which is frequently over-expressed in malignant cells has been chosen as a target for kidney cancer in a study that compare anti-RLIP76 IgG, RLIP76 siRNA, or RLIP76 antisense oligodeoxynucleotide in Caki-2 kidney cancer xenograft bearing Hsd: athymic nude *nu/nu* mice (Singhal et al., 2009). Treatment with RLIP76 antibody, siRNA, or antisense caused regression of established Caki-2 kidney cancer xenografts. mRNA targeting was performed with Quiagen's lipid-based TransMessenger transfection reagent. Caki-2 cells express RLIP76 3-fold compared to normal human kidney mesangial cells. The tumor-bearing animals were alive 4-times longer due to the RLIP76 antibody, RLIP76 siRNA, or RLIP76 antisense treatment. Administration of RLIP76 antibody, siRNA, or antisense caused regression of established Caki-2 kidney cancer xenografts.

4.4.2 Polymer-based delivery

Polymer-based delivery systems have been extensively used for plasmid DNA and more recently for siRNA. As with lipid-based delivery systems, polymeric delivery of siRNA usually involves a cationic moiety as a core component. Cationic polymers are generally classified into synthetic and natural polymers. Synthetic polymers include branched or linear *poly-(etilene-imine) (PEI)*, *poly-(L-lysine) (PLL)*, and *cyclodextrin*-based polycations. Natural cationic polymers include *chitosan, atelocollagen*, and cationic *polypeptides*. Cationic polymers (polyplexes if complexed with DNA) are key players of non-viral transfer systems due to their exclusive physicochemical properties (Kimura et al., 2001). Most important characteristics of non-viral carriers are charge ratio, which define their ability to carry NAs in therapeutically sufficient quantities (Almofti et al., 2003). An extensive research in polymer therapeutics led to new generations of polymers improving safety, biocompatibility, and efficiency. One novel approach aims at glomerular protein knockdown using poly-(ethylene-glycol)-poly-(L-lysine) (PEG-PLL) copolymer-based nanocarriers while avoiding size-selective restraints of the glomerular filter (Shimizu et al., 2010). These polymer nanocarriers have enhanced delivery and retention in the kidney compared to naked siRNA following intraperitoneal administration, more specifically to cells of the glomerulus. Furthermore, the applied PLL carrier proved superior with respect to glomerular targeting when compared to a viral delivery of siRNA. Notably, PEI-siRNA complexes displayed lower renal targeting compared to naked siRNA, however, naked siRNA taken up in the kidney was mostly degraded, whereas renal accumulation of PEI-siRNA resulted in a significantly higher proportion of intact siRNA (Malek et al., 2009).

In vivo pharmacokinetics, tissue distribution and adverse effects studies of PEG-PLL copolymer delivered siRNAs in mice revealed that PEI complexation substantially increased tissue uptake compared to naked siRNA (Malek et al., 2009). Application of naked siRNAs

with almost no uptake of intact siRNA molecules was observed in the kidney, while PEI complexation led to a significant increase in levels of intact siRNAs. PEI(–PEG) complex uptake proved to be composition dependent.

In an other application of PEG-PLL glomerulonephritis was ameliorated by MAPK1 siRNA in MRL/*lpr* lupus nephritis model mice (Shimizu et al., 2010). Fluorescence detection revealed that Cy5-labeled siRNA complexed with PEG-PLL transfected glomeruli successfully, unlike the naked siRNA. MAPK1 mRNA expression in isolated glomeruli was significantly suppressed in mice treated with the MAPK1 siRNA PEG-PLL complexes, whereas control or HVJ-E viral vector mediated siRNA complexes had no effect on protein expression.

Based on a conception that hyaluronic acid (HA) plays an important role on receptor-mediated endocytosis, the effect of HA modification of siRNA/PEI complex has been investigated in B16F1 melanoma tumor-bearing mice (Jiang et al., 2008). The hyaluronic acid conjugated complex exhibited higher gene silencing efficiency in B16F1 murine melanoma cells with HA receptors than the siRNA/PEI complex alone. According to an *in vivo* biodistribution study, siRNA/PEI-HA complex accumulated mainly in tissues with HA receptors such as liver, kidney, and tumor. Anti-VEGF siRNA/PEI-HA complex was used successfully as target specific antiangiogenic therapeutics in the tissues with HA receptors, such as liver cancer and kidney cancer. Intratumoral injection of anti-VEGF siRNA/PEI-HA complex resulted in an effective inhibition of tumor growth by the HA receptor mediated endocytosis to tumor cells in mice.

Cationized gelatin delivered plasmid DNA expressing TGF-β type II receptor (TGF-βRII) complexed by siRNA prevented interstitial renal fibrosis (Kushibiki et al., 2005) in unilateral ureteral obstruction (UUO) model mice.

4.4.3 Aptamers

Aptamers (<lat.> aptus: fit), like antibodies are molecules that bind tightly to their specific molecular targets. Unlike antibodies aptamers can be syntehthised and selected with pure chemical methods, and their production do not involve living systems. Target binding by aptamers is achieved by their 3 dimensional structure.

RNA oligonucleotide aptamers recognize their target specifically on the basis of their unique 3-dimensional structures. Application of aptamers as carriers is based on the specific interaction between the aptamer and its cellular membrane receptor. As aptamers bind their molecular targets like antibodies, internalization of the aptamer enables the cellular uptake via receptor-mediated endocytosis, thereby increasing local concentration of carried drugs in the targeted cells.

Spiegelmer aptamers are l-enantiomers, which are immunologically inert, and are not degraded by nucleases. Ccl2 antagonistic, PEGylated spiegelmer mNOX-E36 aptamer ameliorated diabetic nephropathy in mice (Ninichuk et al., 2008). mNOX-E36–3'PEG reduced the number of glomerular macrophages, significantly improved the glomerular filtration rate, reduced renal Ccl2 mRNA and protein expression, and thus protected from diffuse glomerulosclerosis.

Spiegelmer NOX-F37 aptamer targeting vasopressin-dependent activation of V_{1a} and V_2 receptors, effectively neutralized vasopressin (AVP) and increased diuresis in healthy rats (Purschke et al., 2006).

Aptamer A1, A2 and A3 has been utilized to block AMPA receptor trafficking by inhibiting the glutamate receptor subunit 1 (GluR1) (Liu et al., 2009). Aptamers were selected that

effectively bind to phospho-Ser845 GluR1 protein. This strategy to use aptamers to modify single-residue phosphorylation is expected to facilitate evaluation of the potential role of AMPA receptors in various forms of synaptic plasticity.

4.4.4 Nanoparticles

Various types of *nanoparticles* are used in biomedical research. Few experiments have been already completed for kidney targeting.

- *Nanocrystals* are crystalline structures of aggregated molecules, mostly known as *quantum dots* and are used for biological imaging, semiconductors of material research and chemical engineering.
- *Nanotubes* are self-assembling sheets of atoms (often carbon atoms) arranged in tubes.
- *Fullerenes* are similar to carbon nanotubes in that their molecular framework is entirely composed of an extensive π-conjugated carbon skeleton.
- *Dendrimers* are unique molecular architectures having well defined structures with inner cavities to bind biomolecules that make them appropriate for gene delivery.

Quantum dots (Qdot) were applied for bioimaging purposes in the study, where uptake of siRNA/PEI complexes was enhanced with hyaluronic acid (HA) conjugation (referred earlier in 4.4.2, Jiang et al., 2008).

Nanofibrous scaffold mediated RNAi was applied successfully silencing GAPDH in human embryonic kidney 293 cells (HEK 293) (Cao et al., 2010). Polycaprolactone (PCL) nanofiber encapsulated GAPDH siRNA, and the released intact siRNA from scaffold transfected HEK293 cells.

So far no attempt has been documented for *fullerene-* or *dendrimer-based* RNAi in the kidney, however several gene delivery studies of dendriplexes or fullerenes carrying DNA to other organs were published (Tomalia et al., 2007; Shcharbin et al., 2010; Zhong et al., 2008; Maeda-Mamiya et al., 2010).

Polyamidoamine (PAMAM) dendrimers bound EGFP-C2 marker gene and deliveried it to many organs after intravenous injection, that resulted in high expression in liver, kidney, lung, and spleen (Zhong et al., 2008).

Human embryonic kidney cells (HEK293), mouse embryonic cells (NIH/3T3), SV40 transformed monkey kidney fibroblasts (COS-7) and human epithelioid cervical carcinoma cells (HeLa) were efficiently transfected with dendriplexes carrying pDNA encoding firefly luciferase, beta-galactosidase or green fluorescent protein (Shcharbin et al., 2010).

To investigate a *fullerene* as nanocarrier, tetra(piperazino)fullerene epoxide (TPFE) conjugated Insulin 2 gene coding pDNA was administered to mice (Maeda-Mamiya et al., 2009). Plasmid insertion was more efficient when delivered with fullerene than when delivered with Lipofectin in kidney, liver and spleen. Application of TPFE fullerene carrier did not elevate blood urea nitrogen (BUN), whereas plasmid carried with Lipofectin increased BUN level that indicated a mild kidney toxicity.

5. Conclusion

Nucleic acid therapies reviewed in the present chapter are aimed at silencing messenger RNA. Recent advances in delivery systems may soon advance NA therapy from science fiction to science and medicine, thus enabling therapy of presently uncurable diseases of the kidney such as cancer, or fibrosis.

Delivery method	Carrier	Target RNA	Disease	Model	Functional assays	Author
Hydrodynamic / Lipid	TransIT In Vivo Gene Delivery System, DOTAP	p85α	Acute renal injury	Ischemia-reperfusion	Uptake, biodistribution	Larson et al., 2007
Hydrodynamic / Lipid	Lipofectamine 2000	Fas	Acute renal injury	Ischemia-reperfusion	Blood urea nitrogen, Fas Immunohisto-chemistry, apoptosis, histological scoring	Hamar et al., 2004
Hydrodynamic	n.a.	Apoptosis cascade elements	Acute renal injury	Ischemia-reperfusion	n.a.	Zheng et al., 2008
Hydrodynamic	n.a.	Nuclear factor kappa-b (NFkB)	Acute renal injury	Ischemia-reperfusion	n.a.	Feng et al., 2009
Hydrodynamic /Viral	Lipofectamine 2000	Apoptosis antagonizing transcription factor (AATF)	Acute renal injury	Ischemia-reperfusion	Apoptosis, oxidative stress, caspase activation, membrane lipid peroxidation	Xie & Guo, 2006
Hydrodynamic	pBAsi mU6 Neo/ TransIT-EE Hydrodynamic Delivery System	Gremlin	Diabetic nephro-pathy	Streptozotozin -induced diabetes	Proteinuria, serum creatinine, glomerular and tubular diameter, collagen type IV/BMP7 expression	Q. Zhang et al., 2010
Viral/Lipid	pSUPER vector/ Lipofectamine	TGF-β type II receptor	Interstitial renal fibrosis	Unilateral urethral obstruction	α-SMA expression, collagen content,	Kushibiki et al., 2005
Viral	Adeno-associated virus-2	Mineral corticoid receptor	Hyper-tension caused renal damage	Cold-induced hypertension	blood pressure, serum albumin, serum urea nitrogen, serum creatinine, kidney weight, urinary sodium	Wang et al., 2006
Hydrodynamic /Viral	pU6 vector	Luciferase	n.a.	n.a.	uptake	Kobayashi et al., 2004
Lipid	Lipoproteins, albumin	apoB1, apoM	n.a.	n.a.	Uptake, binding affinity to lipoproteins and albumin	Wolfrum et al., 2007
Lipid	Lipofectamine2000	p53	Acute renal injury	Ischemic and cisplatin-induced acute injury	Histological scoring, apoptosis	Molitoris et al., 2009

Delivery method	Carrier	Target RNA	Disease	Model	Functional assays	Author
Lipid	DOTAP/DOPE, DOTAP/DOPE/ DOPE-PEG2000	COX-2	Breast adeno-carcinoma	MDA-MB-231 breast cancer xenograft-bearing mouse	Cell viability, uptake	Mikhaylova et al., 2009
Lipid	Cholesterol	12/15-lipoxygenase	Diabetic nephro-pathy	Streptozotocin-induced diabetes	Albuminuria, urinary creatinine, histology, type I and IV collagen, TGF-β, fibronectin, plasminogen activator inhibitor 1	Yuan et al., 2008
Lipid	Lipofectamine 2000	Mitochondrial membrane 44 (TIM44)	Diabetic nephro-pathy	Streptozotocin-induced diabetes	Cell proliferation and apoptosis, histology, ROS, mitochondrial import of Mn-SOD and glutathione peroxidase, cellular membrane polarization	Y. Zhang et al., 2006
Hydrodynamic / Lipid	Proteolipo-some	RLIP76	Renal carcinoma	Caki-2 kidney cancer xenograft-bearing mouse	uptake	Singhal et al., 2009
Polymer	PEGylated PEI	Luciferase pGL3	n.a.	n.a.	Uptake, biodistribution, erythrocyte aggregation	Malek et al., 2009
Polymer	PEGylated poly-L-lysine	MAPK1	Lupus glomerulo-nephritis	Glomerulo-nephritis	Proteinuria, glomerulosclerosis, TGF- β, , fibronectin, plasminogen activator inhibitor 1	Shimizu et al., 2010
Polymer/Nano particle	Hyaluronic acid/ Quantum dot/ PEI	VEGF	Kidney cancer/ melanoma	B16F1 melanoma tumor-bearing mouse	Biodistribution, citotoxicity, tumor volume, endocytosis	Jiang et al., 2008
Polymer/Nano particle	PEGylated polycapro-lactone nanofiber	GAPDH	n.a.	n.a.	cell viability, uptake	Cao et al, 2010
Aptamer	Spiegelmer mNOX-E36	CC chemokine ligand 2	Glomerulo sclerosis	Uninephrecto-mized mouse	urinary albumin, urinary creatinine, histopathology, glomerular filtration rate, macrophage count, serum Ccl2, Mac-2+, Ki-67+	Ninichuk et al., 2008

Delivery method	Carrier	Target RNA	Disease	Model	Functional assays	Author
Aptamer	Aptamer NOX-F37	vasopressin (AVP)	Congestive heart failure	n.a.	Binding affinity to D-AVP, Inhibition of AVP Signaling, Urine osmolality and sodium concentration,	Purschke et al., 2006
Aptamer	Aptamer A1, A2, and A3	glutamate receptor subunit 1(GluR1)	n.a.	Human embryonic kidney 293	binding	Liu et al., 2009

Table 1. Application of RNA interference in kidney disease models.

6. References

Almofti, M.R., Harashima, H., Shinohara, Y., Almofti, A., Baba, Y., Kiwada, H. (2003). Cationic liposome-mediated gene delivery: biophysical study and mechanism of internalization. *Arch Biochem Biophys,* (Feb 2003), Vol. 410, No. 2, pp. (246-253), ISSN 0003-9861

Bartel, D.P. (2004). MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell,* (Jan 2004), Vol. 116, No. 2, pp. (281-297), ISSN 0092-8674

Cao, H., Jiang, X., Chai, C. & Chew, S.Y. RNA interference by nanofiber-based siRNA delivery system. (2010). *J Controlled Release,* (Jun 2010), Vol. 144, No. 2, pp. (203-212), ISSN 0168-3659

Caracciolo, G., Pozzi, D., Amenitsch, H. & Caminiti, R. (2005). Multicomponent cationic lipid-DNA complex formation: Role of lipid mixing. *Langmuir,* (Dec 2005), Vol. 21, No. 25, pp. (11582-11587), ISSN 0743-7463

Caracciolo, G., Caminiti, R., Digman, M.A., Gratton, E. & Sanchez, S. (2009). Efficient escape from endosomes determines the superior efficiency of multicomponent lipoplexes. *J Physical Chem. B,* (Apr 2009), Vol. 113, No. 15, pp. (1995-4997), ISSN 1089-5647

Castanotto, D. & Rossi, J.J. (2009). The promises and pitfalls of RNA-interference-based therapeutics. *Nature,* (Jan 2009), Vol. 457, No. 7228, pp. (426-433), ISSN 0028-0836

Cox, D.N., Chao, A. & Lin, H. (2000). piwi encodes a nucleoplasmic factor whose activity modulates the number and division rate of germ line stem cells. *Development,* (Feb 2000), Vol. 127, No. 3, pp. (503-514), ISSN 1011-6370

Feng, B., Chen, G., Zheng, X., Sun, H., Zhang, X., Zhang, Z.-X., Xiang, Y., Ichim, T.E., Garcia, B., Luke, P., Jevnikar, A.M. & Min, W.-P. (2009). Small Interfering RNA Targeting RelB Protects Against Renal Ischemia-reperfusion Injury. *Transplantation,* (May 2009), Vol. 87, No. 9, pp. (1283–1289), ISSN 0041-1337

Gao, K. & Huang, L. (2009). Nonviral methods for siRNA delivery. *Molecular Pharmaceutics,* (May-Jun 2009), Vol. 6. No. 3, pp. (651-658), ISSN 1543-8384

Hamar, P., Song, E., Kökény, G., Chen, A., Ouyang, N. & Lieberman, J. (2004). Small interfering RNA targeting Fas protects mice against renal ischemia-reperfusion injury. *Proc Natl Acad Sci,* (Oct 2004), Vol. 101, No. 41, pp. (14883-14888), ISSN 0027-8424

Jackson, A.L., Bartz, S.R., Schelter, J., Kobayashi, S.V., Burchard, J., Mao, M., Li, B., Cavet, G. & Linsley, P,S. Expression profiling reveals off-target gene regulation by RNAi. *Nat Biotechnol,* (Jun 2003), Vol. 21, No. 6, pp. (635-637), ISSN 1087-0156

Jiang, G., Park, K., Kim, J., Kim, K.S. & Hahn, S.K. (2008). Target specific intracellular delivery of siRNA/PEI-HA complex by receptor mediated endocytosis. *Molecular Pharmaceutics,* (May-Jun 2009), Vol. 6, No. 3, pp. (727-737), ISSN 1543-8384

Juliano, R., Alam, R., Dixit, V. & Ming, X. (2008). Mechanisms and strategies for effective delivery of antisense and siRNA oligonucleotides. *Nucleic Acids Res,* (Jul 2008), Vol. 36, No. 12, pp. (4158-4171). ISSN 0305-1048

Juliano, R., Bauman, J., Kang, H. & Ming, X. (2009). Biological barriers to therapy with antisense and siRNA oligonucleotides. *Molecular Pharmaceutics,* (May-Jun 2009), Vol. 6, No. 3, pp. (686-695), ISSN 1543-8384

Kaucsár, T., Rácz, Zs. & Hamar, P. (2010). Post-transcriptional gene-expression regulation by micro RNA (miRNA) network in renal disease. *Adv Drug Deliv Rev,* (Nov 2010), Vol. 62, No. 14, pp. (1390-1401), ISSN 0169-409X

Kim, S., Garg, H., Joshi, A. & Manjunath, N. (2009). Strategies for targeted nonviral delivery of siRNAs in vivo. *Trends in Molecular Medicine,* (Nov 2009), Vol. 15, No. 11, pp. (491-500), ISSN 1471-4914

Kim, V.N. (2006). Small RNAs just got bigger: Piwi-interacting RNAs (piRNAs) in mammalian testes. *Genes Dev,* (Aug 2006), (Aug 2006), Vol. 20, No. 15, pp. (1993-1997), ISSN 0890-9369

Kimura T, Yamaoka T, Iwase R, Murakami A. (2001) Structure/function relationship in the polyplexes containing cationic polypeptides for gene delivery. *Nucleic Acids Res,* (2001) *Suppl.,* No . 1, pp (203-204), ISSN 0305-1048

Kobayashi, N., Matsui, Y., Kawase, A., Hirata, K., Miyagishi, M., Taira, K., Nishikawa, M. & Takakura, Y. (2004). Vector-Based in Vivo RNA Interference: Dose- and Time-Dependent Suppression of Transgene Expression. *Journal of Pharmacology and Experimental Therapeutics,* (Feb 2004), Vol. 308, No. 2, pp. (688-693), ISSN 0022-3565

Kushibikia, T., Nagata-Nakajimab, N., Sugaib, M., Shimizub, A. & Tabata, Y. (2005). Delivery of plasmid DNA expressing small interference RNA for TGF-b type II receptor by cationized gelatin to prevent interstitial renal fibrosis. *J Controlled Release,* (Jul 2005), Vol. 105, No. 3, pp. (318-331), ISSN 0168-3659

Larson, S.D., Jackson, L.N., Chen, A., Rychahou, P.G. & Evers, B.M. (2007). Effectiveness of siRNA uptake in target tissues by various delivery methods. *Surgery,* (Aug 2007), Vol. 142, No. 2, pp. (262-269), ISSN 0263-9319

Liu, Y., Sun, Q.-A., Chen, Q., Lee, T.H., Huang, Y., Wetsel, W.C., Michelotti, G.A., Sullenger, B.A. & Zhang, X. (2009). Targeting inhibition of GluR1 Ser845 phosphorylation with an RNA aptamer that blocks AMPA receptor trafficking. *J Neurochem,* (Jan 2009), Vol. 108, No. 1, pp. (147- 157), ISSN 0022-3042

Malek, A., Merkel, O., Fink, L., Czubayko, F., Kissel, T. & Aigner, A. (2009). In vivo pharmacokinetics, tissue distribution and underlying mechanisms of various PEI(-PEG)/siRNA complexes. *Toxicology and Applied Pharmacology,* (Apr 2009), Vol. 236, No. 1, pp. (97-108), ISSN 0041-008X

Mikhaylova, M., Stasinopoulos, I., Kato, Y., Artemov, D. & Bhujwalla, ZM. (2011). Imaging of cationic multifunctional liposome-mediated delivery of COX-2 siRNA. *Cancer Gene Therapy,* (Mar 2011), Vol. 16, No. 3, pp. (217-226), ISSN 0929-1903

1 Moazed, D. (2009). Small RNAs in transcriptional gene silencing and genome defense. *Nature,* (Jan 2009), Vol. 457, No. 7228, pp. (413-420), ISSN 0028-0836
3 Molitoris, B.A., Dagher, P.C., Sandoval, R.M., Campos, S.B., Ashush, H., Fridman, E., Brafman, A., Faerman, A., Atkinson, S.J., Thompson, J.D., Kalinski, H., Skaliter, R., Erlich, S. & Feinstein, E. (2009). siRNA Targeted to p53 attenuates ischemic and cisplatin-induced acute kidney injury. *J Am Soc Nephrol,* (Aug 2009), Vol. 20, No. 8, pp. (1754-1764), ISSN 1046-6673
8 Monia, B.P. (1997). First- and second-generation antisense oligonucleotide inhibitors targeted against human c-raf kinase. *Ciba Found Symp.* Vol. 209, pp. (107-119), ISSN 0300-5208
11 Ninichuk, V., Clauss, S., Kulkarni, O., Schmid, H., Segerer, S Radomska, E., Eulberg, D., Buchner, K., Selve, N., Klussmann, S. & Anders, H.-J. (2008). Late onset of Ccl2 blockade with the Spiegelmer mNOX-E36–3'PEG prevents glomerulosclerosis and improves glomerular filtration rate in db/db mice. *Am J Pathol,* (Mar 2008), Vol. 172, No. 3, pp. (628-637), ISSN 0002-9440
16 Purschke, W.G., Eulberg, D., Buchner, K., Vonhoff, S. & Klussmann, S. (2006). An L-RNA-based aquaretic agent that inhibits vasopressin in vivo. *Proc Natl Acad Sci,* (Mar 2006), Vol. 103, No. 13, pp. (5173-5178), ISSN 0027-8424
19 Paroo, Z. & Corey, D.R. (2004). Challenges for RNAi in vivo. *Trends Biotech*, Vol. 22, No. 8, pp. (390-394), ISSN 0167-9430
21 Rácz, Zs. & Hamar, P. (2006). Can siRNA technology provide the tools for gene therapy of the future? *Curr Med Chem,* (Aug 2006), Vol. 13, No 19, pp. (2299-2307), ISSN 0929-8673
24 Rácz, Zs. & Hamar, P. (2008). RNA interference in research and therapy of renal diseases. *Contr Nephrol,* Vol. 159, pp. (78-95), ISSN 0302-5144
26 Saito, K., Nishida, K.M., Mori, T., Kawamura, Y., Miyoshi, K., Nagami, T., Siomi, H. & Siomi, M.C. (2006). Specific association of Piwi with rasiRNAs derived from retrotransposon and heterochromatic regions in the Drosophila genome. *Genes Dev,* (Aug 2006), Vol. 20, No. 16, pp. (2214-2222), ISSN 0890-9369
30 Semizarov, D., Frost, L., Sarthy, A., Kroeger, P., Halbert, D.N. & Fesik, S.W. Specificity of short interfering RNA determined through gene expression signatures. *Proc Natl Acad Sci,* (May 2003), Vol. 100, No. 11, pp. (6347-6352), ISSN 0027-8424
33 Shcharbin, D., Pedziwiatr, E., Blasiak, J. & Bryszewska. M. (2010). How to study dendriplexes II: Transfection and cytotoxicity. *J Control Release,* (Jan 2010), Vol. 141, No. 2, pp. (110-127), ISSN 0168-3659
36 Shimizu, H., Hori, Y., Kaname, S., Yamada, K., Nishiyama, N., Matsumoto, S., Miyata, K., Oba, M., Yamada, A., Kataoka, K. & Fujita, T. (2010). siRNA-Based Therapy Ameliorates Glomerulonephritis. *J Am Soc Nephrology,* (Apr 2010), Vol. 21, No. 4, pp. (622-633), ISSN 1046-6673
40 Singhal, S.S., Singhal, J., Yadav, S., Sahu, M., Awasthi, Y.C. & Awasthi, S.A. (2009). RLIP76: A Target for Kidney Cancer Therapy. *Cancer Res,* (May 2009), Vol. 69, No. 10, pp. (4244-4251), ISSN 0008-5472
43 Siomi, H. & Siomi, M.C. (2009). On the road to reading the RNA-interference code. *Nature,* (Jan 2009), Vol. 457, No. 7228, pp. (396-404), ISSN 0028-0836

Song, E., Lee, S.K., Wang, J., Ince, N., Ouyang, N., Min, J., Chen, J., Shankar, P. & Lieberman, J. (2003). RNA interference targeting Fas protects mice from fulminant hepatitis. *Nature Medicine,* (Feb 2003), Vol. 9, No. 3, pp. (347-351), ISSN 1078-8956

Tseng, Y.-C., Mozumdar, S. & Huang, L. (2009). Lipid-based systemic delivery of siRNA. *Adv Drug Deliv Rev,* (Jul 2009), Vol. 61, No. 9, pp. (721-731), ISSN 0169-409X

Tuschl, T., Zamore, P.D., Lehmann, R., Bartel, D.P. & Sharp, P.A. (1999). Targeted mRNA degradation by double-stranded RNA in vitro. *Genes Dev*, (Dec 1999), Vol. 13, No. 24, pp. (3191-3197), ISSN 0890-9369

Valencia-Sanchez, M.A., Liu, J., Hannon, G.J. & Parker, R. (2006). Control of translation and mRNA degradation by miRNAs and siRNAs. *Genes Dev,* (Mar 2006), Vol. 20, No. 5, pp. (515-524), ISSN 0890-9369

Wang, X., Skelley, L., Cade, R. & Sun, Z. (2006). AAV delivery of mineralocorticoid receptor shRNA prevents progression of cold-induced hypertension and attenuates renal damage. *Gene Therapy,* (Jul 2006), Vol. 13, No. 14, pp. (1097-1103), ISSN 0969-7128

Wolfrum, C., Shi, S., Jayaprakash, K.N., Jayaraman, M., Wang, G., Pandey, R.K., Rajeev, K.G., Nakayama, T., Charrise, K., Ndungo, E.M., Zimmermann, T., Koteliansky, V., Manoharan, M. & Stoffel, M. (2007). Mechanisms and optimization of in vivo delivery of lipophilic siRNAs. *Nature Biotechnology,* (Sep 2007), Vol. 25, No. 10, pp. (1149-1157), ISSN 1087-0156

Xie, J. & Guo, Q. (2006). Apoptosis antagonizing transcription factor protects renal tubule cells against oxidative damage and apoptosis induced by ischemia-reperfusion. *J Am Soc Nephrol,* (Dec 2006), Vol. *17, No. 12, pp. (3336–3346),* ISSN 1046-6673

Yuan, H., Lanting, L., Xu, Z.-G., Li, S.-L., Swiderski, P., Putta, S., Jonnalagadda, M., Kato, M. & Natarajan, R. (2008). Effects of cholesterol-tagged small interfering RNAs targeting 12/15-lipoxygenase on parameters of diabetic nephropathy in a mouse model of type 1 diabetes. *Am J Physiol Renal Physiol,* (Jun 2008), Vol. 295, pp. (F605-F617), ISSN 0363-6127.

Zhang, C. (2009). Novel functions for small RNA molecules. *Curr Opin Mol Ther,* (Dec 2009), Vol. 11, No. 6, pp. (641-651), ISSN 1464-8431

Zhang, Q., Shi, Y., Wada, J., Malakauskas, SM., Liu, M., Ren, Y., Du, C., Duan, H., Li, Y., Li, Y. & Zhang, Y. (2010). In vivo delivery of Gremlin siRNA plasmid reveals therapeutic potential against diabetic nephropathy by recovering bone morphogenetic protein-7. *PloS ONE,* (Jul 2010), Vol. 5, No. 7, e11709, pp. (1-13), ISSN 1932-6203

Zhang, Y., Wada, J., Hashimoto, I., Eguchi, J., Yasuhara, A., Kanwar, Y.S., Shikata, K. & Makino, H. (2006). Therapeutic approach for diabetic nephropathy using gene delivery of translocase of inner mitochondrial membrane 44 by reducing mitochondrial superoxide production. *J Am Soc Nephrol,* (Apr 2006), Vol. 17, No. 4, pp. (1090–1101), ISSN 1046-6673

Zheng, X., Zhang, X., Feng, B., Sun, H., Suzuki, M., Ichim, T., Kubo, N., Wong, A., Min, L.R., Budohn, M.E., Garcia, B., Jevnikar, A.M. & Min, W.P. (2008). Gene Silencing of Complement C5a Receptor Using siRNA for Preventing Ischemia/Reperfusion Injury. *Am J Pathol*, (Oct 2008), Vol. 173, No. 4, pp. (973–980), ISSN 0002-9440

Zhong, H., He, Z.G., Li, Z., Li, G.Y., Shen, S.R. & Li. X.L. (2008). Studies on polyamidoamine dendrimers as efficient gene delivery vector. *J Biomater Appl,* (May 2008), Vol. 22, No. 6, pp. (527-544), ISSN 0885-3282