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REVIEW ARTICLE

siRNA Technology in Kidney Transplantation: Current Status and Future Potential

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Abstract Kidney transplantation is one of the most common transplantation operations in the world, accounting for up to 50 % of all transplantation surgeries. To curtail the damage to transplanted organs that is caused by ischemia–reperfusion injury and the recipient's immune system, small interfering RNA (siRNA) technology is being explored. Importantly, the kidney as a whole is a preferential site for non-specific systemic delivery of siRNA. To date, most attempts at siRNA-based therapy for transplantation-related conditions have remained at the in vitro stage, with only a few of them being advanced into animal models. Hydrodynamic intravenous injection of naked or carrier-bound siRNAs is currently the most common route for delivery of therapeutic constructs. To our knowledge, no systematic screens for siRNA targets most relevant for kidney transplantation have been attempted so far. A majority of researchers have arrived at one or another target of interest by analyzing current literature that dissects pathological processes taking place in transplanted organs. A majority of the genes that make up the list of 53 siRNA targets that have been tested in transplantation-related models so far belong to either apoptosis- or immune rejection-centered networks. There is an opportunity for therapeutic siRNA combinations that may be delivered within the same delivery vector or injected at the same time and, by targeting more than one pathway, or by hitting the same pathways within two different key points, will augment the effects of each other.

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1 Introduction

Kidney transplantation is one of the most common transplant operations in the world, accounting for up to 50 % of all transplantation surgeries. Extending the survival rate of kidney grafts is one of the important priorities of modern medicine. Currently, 1-year first graft survival rates are about 90 % [1, 2]. However, 10-year survival rates are substantially lower (approximately 50 %) and have remained at these percentages despite introduction of novel immunosuppressive and organ-protecting strategies [3, 4]. There is no difference between graft survival rates for organs procured from asystolic donors and grafts explanted from brain-dead donors. However, the rates of delayed graft function and primary non-function are higher in kidneys transplanted from asystolic donors. Warm ischemia time and reperfusion after transplantation are the main factors limiting the viability and quality of grafts from donors with sudden irreversible cardiac arrest.

Both graft dysfunction and acute or chronic rejection prevent substantial increases in long-term graft survival. Loss of function of transplanted organs leads to resumption of kidney replacement therapy in patients, an increase in mortality rates, a decrease in quality of life, and substantial

economic losses [5]. Additionally, patients who have lost their first transplanted organ because of rejection are returned to already overcrowded waiting lists and contribute to the worldwide organ shortage.

A number of pathophysiological processes take place in the graft during its retrieval, storage procedure, and transfer to the recipient. Even immediate transplantation of the organ after procurement results in temporary cessation of blood flow, which, in turn, leads to various degrees of tissue damage due to ischemia–reperfusion injury or the ischemic cascade, which is a complex and damaging process, arising from oxidative stress, mitochondrial integrity impairment, the clotting cascade, and innate and adaptive immune system activation. Ischemia–reperfusion injury is the pathogenic basis of delayed function development, rejection episodes, and primary non-function, mainly through apoptosis and immune response activation in the transplanted organ. Importantly, ischemia–reperfusion injury is detectable not only in organs procured from expanded-criteria donors but also in organs from brain-dead donors [6].

An analysis of clinical predictors of renal allograft histopathology showed that the cold ischemia time is positively associated with the composite Chronic Allograft Damage Index (CADI) score and other histopathological indices of tissue damage [7]. Hence, the severity of ischemia–reperfusion injury may affect subsequent graft function. Moreover, it contributes to delayed graft function, which, in turn, is associated with an increased risk of graft loss [8]. Another driving force that decreases the survival rate of kidney grafts is cellular and/or humoral (antibody-mediated) immune responses, which should be suppressed in order to prevent acute or chronic rejection of the transplanted organ. Lifelong administration of various immunosuppressant drugs leads to an overall increase in patient morbidity, which is not necessarily related to renal function but includes cardiovascular events, malignancies, and increased susceptibility to certain viral infections [9]. Taken together, these factors are major contributors to the limitations in graft survival rates. Therapeutic interventions aimed at diminishing these pathological factors or alleviating their effects would improve the long-term outcomes of kidney transplantation.

To curtail the damage to the transplanted organ that is caused by ischemia–reperfusion injury and the recipient's immune system, a variety of therapeutic approaches are being explored. Small interfering RNA (siRNA) technology has already shown great promise for development of novel formulations aimed at treatment of viral infections, malignancies, macular degeneration, and a variety of other pathologies [10, 11]. The most important advantages of siRNA are its high efficiency in gene silencing and the possibility of developing specific siRNA-based drugs for any gene target, including these encoding protein targets with no known pharmacological antagonists or inhibitors. However, because of the unresolved problem of off-target delivery of siRNA, its clinical applications are limited by the necessity for local access to the target organ or tissue. It seems that isolated organs that are being prepared for transplantation represent a potential niche for siRNA-based gene-targeting approaches.

The kidney is an especially attractive organ target for siRNA and types of gene therapy. To deliver a molecular drug into this organ, a variety of routes are available, including the intravenous route, the intra-arterial route, and the retrograde route through the ureter, or by injections inside the parenchyma [12]. Ex vivo kidney preservation by continuous hypo- or normothermic perfusion at the time of organ storage and transport to the recipient provides a unique therapeutic window when siRNA treatment may be applied directly during the procedure. Moreover, siRNA-based treatments may aim to improve the quality of the graft before surgery. These siRNAs may be added directly to the organ preservation solution, thereby circumventing the need for injection into the bloodstream. Before transplantation, the organ may be washed in order to remove the remaining cell-free siRNA. Therefore, the problems with uptake of siRNA by non-target organs and the cells of the recipient or systemic toxicity will be avoided.

In this review, we concentrate on novel opportunities that have become available with the advent of siRNA technology.

2 Pathological Processes in Grafts and Potential Molecular Targets of siRNA Therapy

In this section, we review pathological processes taking place in the donor kidney before retrieval of the organ, during its storage, and also post-transplantation. Figure 1 depicts the typical sequence of manipulations of donated kidneys.

2.1 Ischemia–Reperfusion

Although transplantation-related injury of kidney grafts was originally thought to be the result of ischemia alone, it mainly occurs during the period of reperfusion. Ischemia–reperfusion is a multifactorial inflammatory condition, which builds up through synergy between an increase in the permeability of the endothelium and its activation, recruitment and infiltration of leukocytes, their adhesion to each other and to platelets, and the release of reactive oxygen species and inflammatory cytokines, enhancing the inflammatory reaction [13]. Together, these processes lead to depletion of adenosine triphosphate (ATP), the shift of

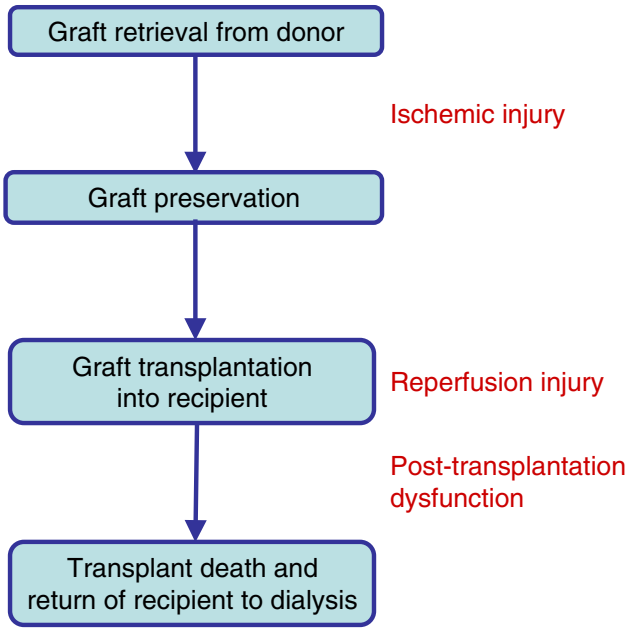

Fig. 1 General scheme of injuring events during transplantation

ionic homeostasis, and subsequent cell death (Fig. 2). In the hypothermic preservation that is typical of transplantation, the marked increase in production of free radicals augments the damage [14]. The sum of these injuries contributes to early graft dysfunction or a delay in its function, which, in turn, promote chronic dysfunction and compromise outcomes.

The severity of initial ischemia depends on the pathophysiological processes within the donor body, mainly hemodynamic disturbances related to the cause of death. When the blood supply is restricted, metabolic pathways are deprived of energy influx, while the end products accumulate, thus resulting in profound impairment of mitochondrial metabolism [15]. Upon connection of the kidney graft to the bloodstream of the recipient, reperfusion and subsequent reoxygenation of its tissues lead to an excessive supply of electrolytes, oxygen, water, glucose, and other substrates. The impaired graft tissues, which are confounded with background mitochondrial dysfunction, are unable to cope with such intensive metabolic pressure. Subsequently, necrotic, apoptotic, and autophagic cascades are initiated. Hence, reperfusion is an effector phase of ischemic injury, which makes the greatest contribution to ischemia/reperfusion damage [16, 17]. Importantly, the processes of tissue regeneration and recovery take place on the same timescale as cellular death. The fate of the transplanted organ depends on the interplay of these two processes.

Leukocyte activation is an important mechanism of kidney damage during ischemia [18]. The resident macrophages and dendritic cells recognize overproduction of adhesion molecules, such as P- and E-selectins, as an activation of endothelial cells, and migrate towards the ischemic area. Within the hypoxic tissue, leukocytes initiate a nonspecific inflammatory response regulated mainly by T and B lymphocytes. Since the surfaces of these cells present antigens, accumulation of lymphocytes in the kidney graft may result in transplant rejection. The neutrophils are the first cells to enter the kidney parenchyma. These cells release free radicals, reactive nitrogen species, and cytokines to perpetuate the vicious cycle of inflammation, which attracts other peripheral leukocytes to the affected organ [19, 20].

Another important consequence of ischemia is complement activation, which occurs via the alternative pathway at the outer basolateral surface of the proximal tubules of a transplanted kidney [21, 22]. It involves local synthesis of several key factors of the complement system and denudation of membrane-bound regulators, thus restricting activation of the main C3 component [23]. Importantly, a significant increase in renal expression of complement genes is registered in kidney grafts prior to their reconnection with a bloodstream [24], while deposition of the complement occurs mostly during the reperfusion phase. This provides a rationale for possible siRNA-based anti-complement preconditioning of the graft prior to its transfer to the patient.

2.2 Other Relevant Post-transplantation Events

The most common types of post-transplantation dysfunction include primary non-function and early allograft nephropathy, chronic allograft nephropathy, and delayed graft function (Fig. 3).

2.2.1 Primary Non-function and Early Allograft Nephropathy

Primary non-function is defined as permanent loss of function starting immediately after transplantation. It accounts for about 3 % of all cases of renal allograft failure [25]. Any deterioration of kidney graft function within 3–6 months post-transplantation is defined as early allograft nephropathy [26].

Common causes of initial kidney graft non-function and early allograft nephropathy range from early acute or subclinical cellular and humoral rejection to acute tubular necrosis, calcineurin inhibitor nephrotoxicity, BK virus nephropathy, and recurrent glomerulonephritis [27]. siRNA therapeutic interventions for these causes could be conceived; however, the treatment window is short, especially in the case of primary non-function. It seems that the best treatment for primary non-function may be its prevention by siRNA-based treatments aimed at improving the quality of the graft before surgery, which, in turn, would provide better therapeutic outcomes.

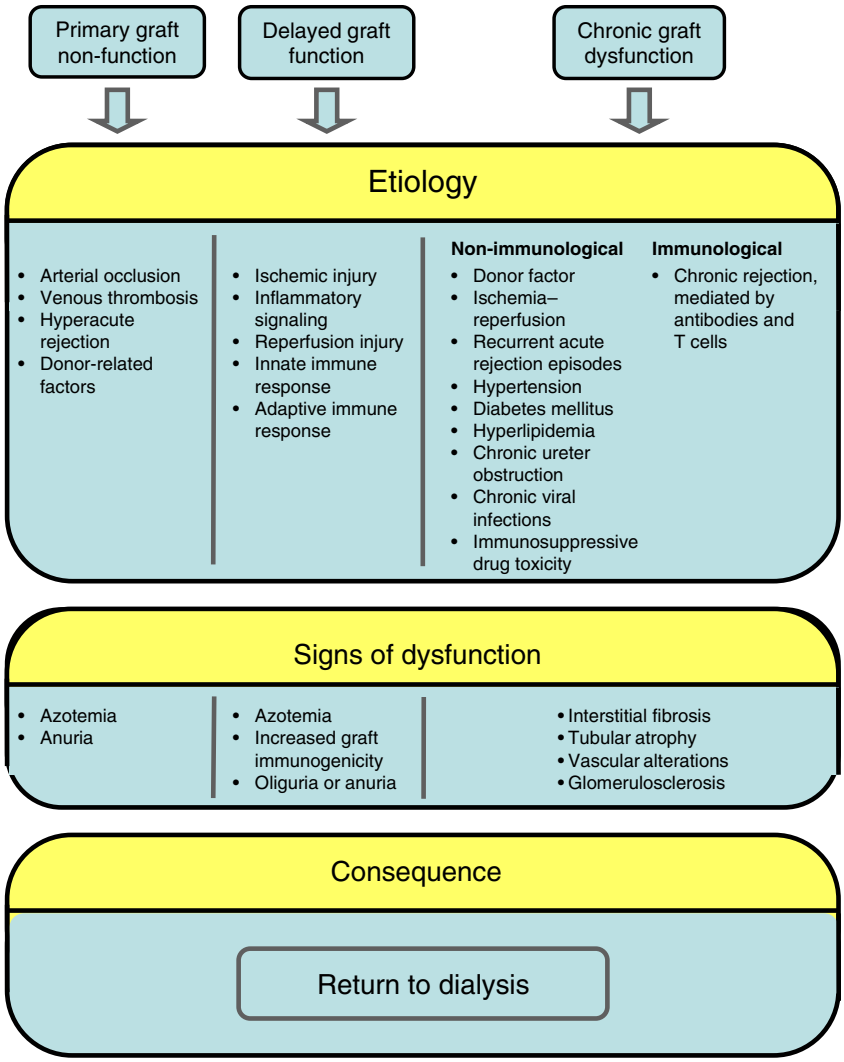

Fig. 2 Post-transplantation dysfunction

2.2.2 *Delayed Graft Function*

Delayed graft function is a form of acute renal failure, which manifests as post-transplantation oliguria, requiring dialysis, and an increase in allograft immunogenicity, which may lead to an acute rejection episode [28]. Delayed graft function is the most common complication post-transplantation. Depending on the type of donor, it occurs in up to 57 % of kidneys transplanted from asystolic donors and in only 21 % of kidneys retrieved from brain-dead donors [29].

Delayed graft function may be caused by a variety of factors and may be related to conditions in the donor or the recipient, as well as to organ procurement and storage. Delayed graft function affects outcomes for the graft and also the long-term survival of the patient [8]. Both immune and non-immune mechanisms contribute to delayed graft function, with ischemia–reperfusion playing a significant role [30]. Importantly, histological studies of grafts delayed in their function have not revealed substantial damage to the kidney parenchyma [31], thus raising hope that timely molecular intervention may help to overcome the delay in function.

In the timeframe of the days to weeks after transplantation, the regenerating tubular epithelium and peritubular capillary network could use support. Nevertheless, except for calcium-channel blockers [32], no specific pharmaceutical interventions have been developed for prevention or alleviation of ischemia–reperfusion in kidney grafts. siRNA-based therapies may aid recovery of the graft from ischemia–reperfusion injury to avoid interstitial fibrosis and permanent loss of nephrons.

2.2.3 *Chronic Allograft Nephropathy*

Chronic allograft nephropathy is a generic term that covers all causes of chronic renal allograft dysfunction characterized by gradual worsening of renal function, arterial hypertension, and low-grade proteinuria. Histologically, chronic graft dysfunction manifests as interstitial fibrosis

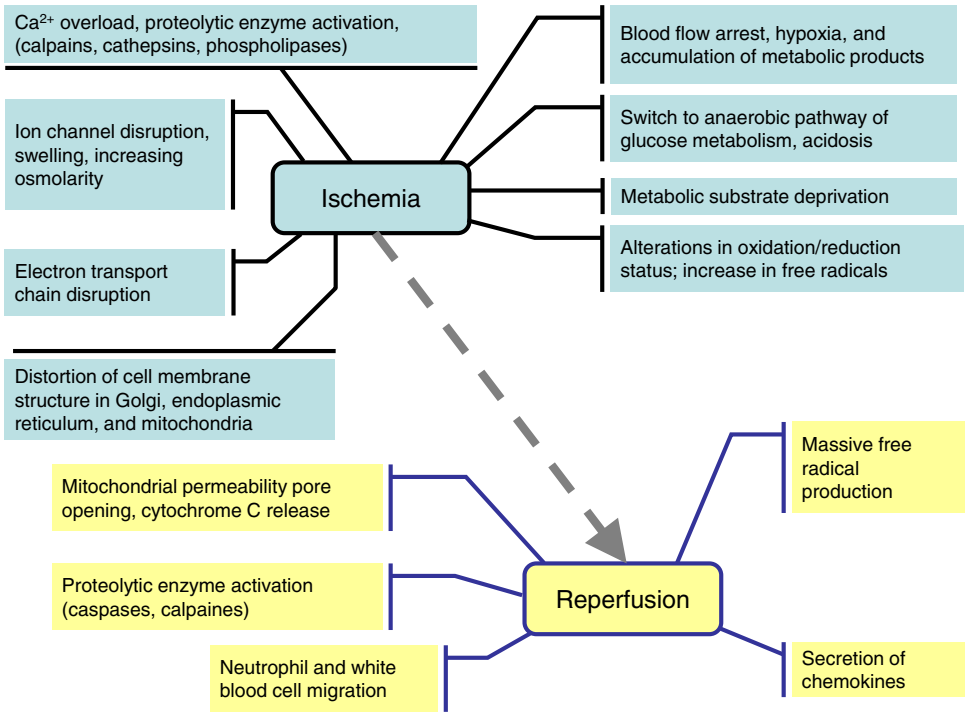

Fig. 3 Consequences of ischemia–reperfusion

and tubular atrophy accompanied by glomerulosclerosis [33]. It may be due to immunological or non-immunological causes, the latter including drug toxicity, bacterial or viral infections, an increase in blood pressure, obstruction, or recurrent or de novo renal disease. In most cases, the most common histological finding in dysfunctional kidneys is a combination of persistent inflammation, interstitial fibrosis, and tubular atrophy, with myofibroblasts being the key—albeit not exclusive—effector cells in renal fibrogenesis, resulting in upregulated extracellular matrix synthesis [34]. In biopsy samples from renal transplant recipients with allograft nephropathy due to interstitial fibrosis and tubular atrophy, the levels of messenger RNA (mRNA) encoding the profibrotic factors connective tissue growth factor (CTGF), kidney injury molecule (KIM)-1, and transforming growth factor (TGF)-β are increased [35, 36]. Among these factors, TGF-β is the most studied one; consequently, treatments aimed at its abrogation are the most common theme in translational research to curtail delayed graft function [37, 38]. TGF-β suppression by small hairpin RNA (shRNA) is being studied in animal models of allograft fibrosis [39] and obstruction-related chronic kidney diseases [40], with promising results.

3 Animal Models of Kidney Transplantation and Their Suitability for siRNA Studies

Studies of siRNA-based therapeutics aimed at organ transplant applications are commonly performed in cell lines in vitro and in animal models in vivo. The obvious disadvantages of the cell-based models limit their usefulness to studies of the efficiency of target gene suppression. Many researchers skip cell-line testing in its entirety and elect to perform studies in vivo. However, it should be noted that animal experimental models are not always adequate. Most commonly employed models include mice and rats, which provide an opportunity to investigate potential therapeutics, but the typical results of such studies cannot be extrapolated to humans. The studies using larger animals, such as primates or porcine models, are of great preclinical value, but they are extremely rare and limited because of their associated costs.

To evaluate potential siRNA therapeutics, one should ideally aim to evaluate various clinically relevant, longitudinal measures of transplantation success in rat, mouse, or porcine models while comparing siRNA-treated and non-treated study arms. However, most often, researchers concentrate on the study of specific pathophysiological process such as ischemia–reperfusion injury [41], acute allograft rejection [42], or chronic allograft nephropathy [39]. A wide variety of different models of acute allograft rejection have been presented by different study designs, thus making adequate comparison of different siRNA implementation scenarios difficult.

To date, most attempts at siRNA-based therapy for transplantation-related conditions have remained at the in vitro stage, with only a few of them being advanced into animal models.

3.1 Warm and Cold Ischemia Models

Because of its relative simplicity, the rodent model of warm ischemia–reperfusion has become one of the most popular ways to study the potential of siRNA in kidney transplantation. The technique includes clamping of the renal pedicle for varying time periods, usually from 15 to 45 min (so-called warm ischemia), then removal of the clamp, followed by reperfusion. Warm ischemia commonly occurs during living kidney donation and transplantation [43], and it is therefore relevant to clinical settings. Table 1 outlines the details of different warm ischemia models and includes typical results of the studies of siRNA efficiency in these models. As can be seen from this table, in warm ischemia–reperfusion models, commonly used means of siRNA delivery include expression of shRNA from U6.1 promoter plasmids and infusion of naked DNA. Among the typically measured outcomes are the levels of nitrogen in urea, the size of infarction, and very early endpoints of post-surgical survival (days). Among the molecular endpoints are expression levels of the target protein [44] or its downstream regulatory targets [45].

It is important to note that in typical clinical settings, the removed kidney is preserved in static cold storage. This technique remains the most popular method of kidney graft preservation because of its simplicity and low cost. A number of studies have evaluated the effects of siRNAs in a cold storage model, the typical means of delivery being via an intravenous or intra-arterial route with subsequent clamping [41, 46, 47]. Table 2 provides a detailed description of studies focusing on cold ischemia models (Table 2).

3.2 Models of Post-transplantation Kidney Injury

There are several pathological conditions that may potentially lead to graft dysfunction, including acute kidney graft injury, acute renal graft rejection, and chronic allograft nephropathy. The accumulated body of knowledge that describes the molecular underpinnings of these conditions has opened up an avenue for development of relevant siRNA-based therapies. For example, acute injury processes in kidney grafts are very similar to those in normal kidneys, and a range of biomarkers are available for diagnostics and monitoring of this condition [48]. Molitoris and co-authors [49] tested siRNA targeted at p53, a pivotal protein in the apoptotic pathway, to prevent kidney injury in a rat model, with encouraging results. As acute kidney injury is usually connected to infectious conditions, specific siRNA targeting of T cells looks promising to reduce inflammation [50]. Chronic allograft nephropathy is caused by a complex pathophysiological interplay between chronic rejection, inflammation, and perpetuation of tissue remodeling, and leads to loss of the function of the graft due to fibrosis [51, 52]. In several rat models, beneficial effects of siRNA-based suppression of TGF-β1 and CTGF have been demonstrated [39, 53]. Additionally, a recent attempt at treatment of acute humoral kidney graft rejection by siRNA targeted at CD40 allowed a switch of the rejection from the humoral to the cellular type. Moreover, CD40 silencing reduced donor-specific antibodies, graft complement deposition, and immune–inflammatory mediators [43].

4 Advantages and Limitations of siRNA Delivery into the Kidneys

When selecting a siRNA delivery vector, the structure and the tissue composition of the target organ should be taken into account. The rate of the blood flow in the kidney is 400 ml/100 g of tissue per minute; this is much greater than that of other organs—for example, 100 ml/100 g per minute in the liver. The kidney is intricately woven from a few histologically distinct types of tissue and includes at least 26 terminally differentiated types of cell with low mitotic activity [54]. These cells make up the cortical and juxtamedullary nephrons, blood vessels, and the interstitium. The glomerular basement membrane forms the boundary between the blood and the urine. Within the glomeruli, the glomerular basement membrane and the slits between the podocytes perform the filtration that separates the blood in the capillaries from the filtrate formed in Bowman's capsule.

The glomeruli comprise the key tissue compartment for inflammatory processes within the kidney. Hence, it is logical to target them with siRNA therapy. However, the anatomical compartmentalization of the glomeruli is an important consideration for potential gene therapy, as it may preclude access to certain types of target cells. To penetrate this barrier, a number of approaches have been developed, including hydrodynamic injections and use of electric fields and ultrasound. These methods are aimed at barrier penetration without barrier destruction or damage to its constituents. Delivery of therapeutic compositions to the glomeruli is also limited by the small size of the glomerular basement membrane pores, which is 4 nm, while the pores in the endothelial lining of the typical blood vessel are between 70 and 100 nm. However, though direct delivery of large supramolecular complexes through the glomerular basement membrane is precluded, therapeutic siRNA-containing complexes may attach to mesangial cells, which, in turn, have access to the endothelium [55].

So far, there are no specific techniques that may allow siRNA transfer into the nephron tubules. The simplest way to reach the tubules in a non-specific way is by

Table 1 Studies of potential small interfering RNA (siRNA) therapeutics in ischemia–reperfusion models

Model animal	Induction method	Target	Target cells	siRNA and carrier type	Delivery method	Injection time	Dose	Results as compared with control animal group
Mouse [44]	Renal vein and artery clamping for 15 or 35 min	Fas	Tubule cells	Naked	HDI, low-volume injection in vein, or both	48 h before IRI or 2 days after IRI	50 μg	Urea nitrogen level ↓ 53 %, infarction size ↓ 44 %, survival rate 2 days after surgery ↑ 60 %, Fas+ cells in tubules ↓ 3.8-fold (first day after surgery), apoptotic cells on second day after IRI ↓ 2-fold
Rat [45]	Renal artery clamping for 45 min	IKKβ	Not specified	Naked	Renal artery injection	48 h before IRI	0.25 mg/kg	Urea nitrogen and serum creatinine levels ↓ 69 and 81 %, respectively, infarction size ↓ 44 %, NF-κB with DNA binding inhibition, macrophage infiltration limited 2.2-fold, IL-18 and NGAL expression ↓ 2.6-fold
Mouse [88]	Renal vein and artery clamping for 25 or 35 min	RelB	Not specified	Expression vector PRNATU6.1/ Neo	HDI in tail vein	48 h before IRI	50 μg	Urea nitrogen and serum creatinine levels ↓ 37 and 48 %, respectively, cast formation and leukocyte infiltration disappearance, infarction size ↓ 57 %, edema ↓ 67 %, same level of tubule vacuolization, significant ↓ in TNF-α expression, 8-day survival ↑ 71 %
Mouse [89]	Renal vein and artery clamping for 25 or 35 min	Caspase 3 and caspase 8	Not specified	Expression vector PRNATU6.1/ Neo	HDI in tail vein	48 h before IRI	50 μg	Urea nitrogen and serum creatinine levels ↓ 56 and 64 %, respectively, 8-day survival ↑ 49 %
Mouse [90]	Renal vein and artery clamping for 25 min	Caspase 3 and complement component C3	Not specified	pRNAT-U6.1/Neo	HDI in tail vein	48 h before IRI	50 μg	Urea nitrogen and serum creatinine levels ↓ 61 and 75 %, respectively, infarction size ↓ 65 %, tubule damage, vacuolization, cast formation, and leukocyte infiltration were significant, 8-day survival ↑ 70 %
Mouse [91]	Renal vein and artery clamping for 25 or 30 min	Complement component C3	Not specified	Expression vector PRNATU6.1/ Neo	HDI in tail vein	48 h before IRI	50 μg	Urea nitrogen and serum creatinine levels ↓ 59 and 64 %, respectively, infarction size ↓ 71 %, leukocyte infiltration ↓ 50 %, edema ↓ 60 %, cast formation ↓ 92 %, same level of tubule vacuolization, IRI-induced expression of TNF-α mRNA ↓ 8.3-fold, 8-day survival ↑ 49 %
Mouse [92]	Renal vein and artery clamping for 25 or 30 min	C5a receptor	Not specified	Expression vector pRNAT-U6.1/ Neo	HDI in tail vein	48 h before IRI	50 μg	Urea nitrogen and serum creatinine levels ↓ 61 and 79 %, respectively, IRI-induced expression of TNF-α and chemokines MIP-2 and KC4 ↓ 2.2- and 1.7-fold, respectively, infarction size ↓ 65 %, neutrophil infiltration ↓ 66 %, cast formation ↓ 70 %, same level of tubule vacuolization, 8-day survival ↑ 59 %
Mouse [93]	Renal vein and artery clamping for 30 min	Sphingosine-1-phosphate receptor	Tubule cells	siSTABLE naked	HDI in tail vein	48 h before IRI	50 μg	Serum creatinine level ↓ 39 %, infarction size ↓ 60 %, apoptotic cell numbers ↓

HDI hydrodynamic injection, *IL* interleukin, IRI ischemia–reperfusion injury, *MIP* macrophage inflammatory protein, *mRNA* messenger RNA, *NF* nuclear factor, *NGAL* neutrophil gelatinase-associated lipocalin, *TNF* tumor necrosis factor

Table 2 Cold ischemia model

Object	Method	Target	Target cells	siRNA carrier	Delivery method	Injection time	Dose	Result (animal control groups)
Rat [46]	Autotransplantation (warm ischemia): left kidney transplantation into rats after nephrectomy 40 min after intentional warm ischemia; singenic (cold ischemia model) combination of 5 h cold and 30 min warm graft ischemia	p53	Proximal tubule cells	Naked with phosphodiesterase frame modification	100 µl bolus intravenous injection	*Cold ischemia*: donor 30 min before nephrectomy, recipient − 15 min or 4 h post-transplantation	12 mg/kg	*Cold ischemia:* after 1 day, serum creatinine ↓ 44 %; this ratio was maintained for 1 week
Rat [76]	Left kidney was retrieved, infused with UW solution, stored on ice for 30 min, then transplanted to left-nephrectomized recipient	SHARP-2 control expression of IL-2 and IFN-γ)	Not specified	shRNA with lentivirus-based delivery system, LV-SHARP-2iC		30 min ice storage	5×10^7 TU construct in 150 ml UW solution	*SHARP-2* expression ↓ 61 %, *IL-2* ↓ 47 %, *IFN-γ* ↓ 58 %, survival time ↑ 1.4-fold
Porcine kidney [94]	10 min warm ischemia, 24 h ice storage followed by 3 h machine perfusion at 4° C with autologous oxygenated blood [Hosgood, 2010 method]	Caspase 3	Not specified	Naked	Renal artery injection with clamped renal vein, then autologous blood perfusion	During ice storage	3 µg g/ml hyperosmolar citrate solution 0.15 µg g/ml hyperosmolar citrate solution	Cells with active caspase 3 cells ↓ 40 %, apoptotic cells in kidney cortex ↓ 47 %, inflammatory cells ↓ 26 %, oxygen consumption ↑ 2-fold, significant pH neutralization of perfusate
Mini-pig [95]	Left nephrectomy, then UW solution infusion followed by 24 h ice storage; left kidney allotransplantation after right nephrectomy	Caspase 3	Not specified	Naked	Renal artery injection with renal pedicle clamped		0.3 mg/pig (≈11 µg/kg in UW solution	At 48 h, caspase 3 mRNA expression ↓ 83 %, 32 kDa precursor ↓ 54 %; in kidneys treated with siRNA, caspase 3 mRNA expression level ↑ 2.4-fold, 17 kDa precursor ↑ 2.2-fold, 32 kDa precursor ↓ 3.9-fold; cells with active caspase after conservation ↓ 3-fold, with 2.3-fold ↑ post-transplantation; apoptotic cells ↑ 4-fold post-transplantation in control group and 17-fold after siRNA application, MPO+ cells ↑ 4-fold in control group and ↑ 11-fold after siRNA application; damage zone ↓ 18 % after conservation but ↑ 20 % post-transplantation vs control group, creatinine and urea nitrogen levels after conservation not affected; in siRNA-treated grafts, post-transplantation creatinine levels ↑ 32 %, urea nitrogen ↑ 43 %

IFN interferon, *IL* interleukin, *IV* intravenous, *MPO+* myeloperoxidase-positive, *mRNA* messenger RNA, *SHARP* split- and hairy-related protein, *shRNA* small hairpin RNA, *siRNA* small interfering RNA, *TU* transforming units, *UW* University of Wisconsin

hydrodynamic injection or low-volume injection of naked siRNAs into the renal vein. It is assumed that these siRNAs then transduce the parenchymal cells of the proximal tubules [44]. The siRNAs could also be directly injected into the parenchyma or introduced into the kidney through the ureter or the renal artery [56].

Tubulointerstitial inflammation and fibrosis are common components in the majority of progressive renal diseases [57, 58]. Therefore, targeting of siRNA at the interstitium is of paramount importance. Among the proposed techniques for interstitial delivery of siRNA are delivery to the renal artery with venous clamping; hydrodynamic delivery through the renal vein; retrograde access through the ureter; and ultrasound with microbubbling or electroporation. The packaging of siRNA can be either nonexistent (naked siRNA) or as complex as it gets—for example, it may involve wrapping siRNA in hemagglutinating virus of Japan (HVJ)-based liposomes or trapping genetically modified macrophages in the inflamed kidneys (see [57] for review). Most of these approaches were actually developed for the delivery of plasmid DNA or single-strand antisense oligodeoxynucleotides; however, they are equally suitable for delivery of siRNA to the same target tissue [59].

Importantly, the kidney as a whole is a preferential site for non-specific systemic delivery of siRNA. In that respect, the kidneys are similar to the liver. It is generally accepted that the relative availability of the kidney is due to its role in elimination of circulating metabolites and their reabsorption in nephrons. In the kidneys, siRNAs are absorbed in the same manner as other macromolecules with a molecular mass of less than <50 kDa and a diameter of less than 6 nm. These molecules are filtered by the glomeruli, followed by either excretion via the urine or, to a lesser degree, absorption by the cells in the tubules [60].

Kidney filtration is responsible for fast elimination of siRNAs from plasma. In fact, studies of a siRNA-based molecular drug, I5NP, in rodents and non-human primates showed that its intravenous delivery resulted in very rapid clearance with predominant distribution to the kidney, with very low levels being delivered to the liver and other tissues [61].

5 Practical Notes on siRNA Delivery to Kidney Cells in Vivo

5.1 Naked siRNA

The current literature describes a fair number of various techniques for specific siRNA delivery into various organs and tissues, both in vitro and in vivo [62, 63]. However, only a few of them have been thoroughly tested for delivery into the kidneys in vivo. In rodent models, naked siRNA preparations have most often been delivered hydrodynamically, through the tail vein. In some studies, injected siRNAs were not protected from enzymatic digestion [41, 44]; in others, they were protected by chemical modification—for example, 2′O-methylation [49] or changes to the negatively charged phosphodiester backbone [46]—or commercially available nuclease-resistant siSTABLE siRNA was used [64, 65].

Interestingly, the only siRNA preparation approved for phase II trials for treatment of renal disease, I5NP, is a naked siRNA preparation. This molecular drug is being developed by Quark Pharmaceuticals (Fremont, CA, USA) to protect cells from acute ischemia–reperfusion injuries, such as the acute kidney injury that can occur during major cardiac surgery and delayed kidney graft function [61]. I5NP is delivered intravenously and targets the pro-apoptotic gene tumor protein P53 (*TP53*) [66].

5.2 shRNA and siRNA-Encoding Plasmids

An alternative to naked siRNA delivery is a plasmid that may express either shRNA or siRNA. Delivery of the plasmid usually results in a more stable knockdown effect than direct delivery of siRNA molecules. siRNA-expressing plasmids are tandem-type vectors, which transcribe a pair of sense and antisense RNAs initiated from individual promoters. shRNA-encoding plasmids direct a transcription of a single-strand RNA that transcribes a single-strand RNA that forms hairpin, which is processed into siRNA inside cells [67]. For shRNA, the efficiency of the knockdown efficiency is generally higher than that of tandem-type vectors [68].

The choice of the promoter is extremely important for both the levels and the duration of RNA interference effects, and also for the efficiency of the target gene knockdown [68, 69]. The most commonly used promoters are these recognized by RNA polymerase III (Pol III), such as small nuclear RNA U6 (U6) and human RNase P RNA H1 (H1), the latter mediating more stable effects. Another approach is to use Pol II promoters, which can provide cell- or tissue-specific expression of siRNA.

As compared with direct delivery of siRNA, shRNA- or siRNA-expressing plasmid DNAs and siRNA have advantages and disadvantages. Among the advantages of plasmid delivery are the possibility of achieving constitutional expression, and, in the case of Pol II-guided expression, tissue specificity. Among the disadvantages, one may list the necessity for nuclear delivery of the construct that has to be transcribed and the resultant RNA being exported back to the cytosol, where it is processed to siRNA. This disadvantage is especially important for poorly proliferating cells with low permeability of the nuclear envelopes.

The shRNA approach was successfully tested in Anti-Thy-1–treated rats, which developed subsequent glomerulonephritis. The pathological changes in the nephritic kidney were ameliorated by suppression of production of the fibrogenic factor TGF-β1 [70, 71]. Another group was able to slow down the progression of renal interstitial fibrosis by retrograde injection of siRNA-expressing plasmid DNA complexed with cationized gelatin; in this case, the molecular drug was aimed at local suppression of the TGF-β receptor [72]. Importantly, adding the gelatin, a denatured form of collagen, was crucial to the efficiency of the treatment. The authors speculated that the plasmid DNA–gelatin complexes retrogradely injected via the ureter may easily infiltrate into the interstitial area by slipping through between ureteric epithelial cells, and may subsequently distribute in the cortical interstitial space by simple diffusion [72]. Du and co-authors used shRNA-based therapy targeting Fas and caspase 8 to protect the kidneys from ischemia–reperfusion-induced injury after subhypothermic clamping of the renal artery in uninephrectomized mice. In renal tubular epithelial cells from treated animals, both Fas and pro-caspase 8 expression levels were significantly knocked down, resulting in resistance to apoptosis and protection of the kidney as indicated by reduction of renal tubular injury, serum creatinine, and blood urea nitrogen [73].

5.3 Viral Delivery

Viral vectors are efficient agents for delivery of siRNA that can be also made tissue specific. Because of the low mitotic activity of kidney cells, the lentiviral vectors are the most popular, as they are able to transduce post-mitotic cells [74]. For example, Zhou and co-authors successfully tested lentivirus-mediated shRNA targeting collagen type I in both cultured rat mesangial cells and by renal parenchyma injection [75]. Another group showed that lentivirally delivered shRNA against split- and hairy-related protein (SHARP)-2, which controls expression of interleukin (IL)-2 and interferon (IFN)-γ—both of which both play a key role in transplant rejection—could prolong the survival of kidney grafts in a rat model. Importantly, the latter study used perfusion of isolated organs [76].

To date, the prospects for clinical use of lentiviral-based vectors remain unclear, as it would raise a number of safety issues, including the possibility to generate replication-competent recombinant lentivirus either during vector production or in the process of interaction with endogenous retroviruses in the bodies of patients, insertional mutagenesis, and alteration of germline cells with resultant inheritance and dissemination in offspring. Additionally, lentiviruses might stimulate the immune system in a TLR3/TLR7-dependent manner [77]. Some of these features have been alleviated with advancements in construction of the new generation of lentiviral vectors. Further modifications and safety precautions may be required before lentiviral delivery of shRNA can proceed to clinical use.

5.4 Other Approaches for siRNA Delivery

In recent years, a number of synthetic carriers have been specially developed or modified for delivery of chemically synthesized siRNA. The most advanced of them not only ensure penetration through cell membranes but also enhance specificity through selective accumulations in targeted tissues and facilitated intracellular trafficking by utilizing targeting moieties and cell-penetrating peptides.

So far, there have been only a handful of attempts to use synthetic carriers for specific delivery of siRNA into the kidneys. For example, in a mouse model of lupus glomerulonephritis, Shimizu and co-authors used poly(ethylene glycol)-poly(L-lysine)-based vehicles forming siRNA-containing complexes that were small enough to penetrate the fenestrated endothelium and access the mesangium. Molecular drug against mitogen-activated protein kinase 1 (MAPK1) was delivered into the peritoneum to improve kidney function, reduce proteinuria, and ameliorate glomerular sclerosis [78]. A successful attempt at transplantation-relevant delivery into renal tubular epithelial cells used siRNA wrapped up in a linear polyethylenimine derivative, jetPEI. In their study, Li and co-authors suppressed paired box 2 (PAX2) re-expression and remitted renal interstitial fibrosis in a rodent model of obstructive nephropathy [79].

A very intricate technology for podocyte-specific delivery was developed by Hauser and co-authors [80], who generated an anti-mouse podocyte antibody, cleaved into monovalent antigen-recognizing fragments and linked to a NeutrAvidin molecule, which was further connected to biotin-conjugated protamine, a polycationic nuclear protein and universal adaptor for anionic siRNA. The tail vein injection of this delivery system, named shamporter (sheep anti-mouse podocyte transporter), into normal rats substantially reduced the protein levels of cargo siRNA-targeted nephrin or TRPC6. In the event of a need for therapeutic delivery to podocytes, these model targets may be replaced by any other gene. Importantly, monovalent antibodies used as targeting components of the shamporter do not activate complement, a very important advantage confirmed by immunofluorescent staining of rat tissues with C5b-9 and C3 components [80]. Unfortunately, the limitation of this system is that the targeting component for therapeutics intended for human use would have to be developed from scratch.

Recently, some substantial progress has been achieved in the targeting of siRNA at various cell types and tissue compartments by harnessing siRNA carriers with

antibodies to specific membrane components of these cells. For example, targeted liposomes based on the cationic amphiphile SAINT-C18 (1-methyl-4-(cis-9-dioleyl)methyl-pyridinium-chloride), or SAINT-O-Somes for short, were aimed at inflamed endothelial cells by fusion with antibodies against vascular cell adhesion protein 1 (VCAM-1). These formulations, devoid of liver and kidney toxicity, were used for siRNA-based knockdown of VE-cadherin and nuclear factor (NF)-κB p65 mRNAs in inflamed renal microvasculature [81]. One can easily imagine that similarly designed conjugated particles can be employed in targeting of other types of renal cells—for example, tubular epithelium.

Some studies have exclusively concentrated on technical aspects of carrier-based delivery to the kidneys rather than on therapeutically relevant endpoints. Biodistribution of intravenously injected dextran nanogel particles loaded with siRNA was investigated in mice. An accumulation of particles in the kidneys was observed immediately after the injection, while transition of siRNA from the kidney to the bladder was significantly delayed at the glomerular filtration barrier, in contrast to injection of free siRNA [82]. This observation indicates that systemic delivery of loaded nanoparticles may result in substantial preference for accumulation in the kidneys.

5.5 Routes for Delivery of siRNA into the Kidney

Hydrodynamic intravenous injection of naked or carrier-bound siRNAs is currently the most common route for delivery of therapeutic constructs into rodent models of kidney pathologies (see Tables). Despite that, most of the models in studies of systemic delivery by hydrodynamic injections were aimed at suppression of gene targets within the liver parenchyma. Injection of a therapeutic solution volume of about 10 % of the animal body mass takes from 15 to 30 s in rats [83] and 5–7 s in mice [84]. It is assumed that the resultant hypervolemia leads to a rapid increase in pressure in the inferior vena cava, followed by enlargement of fenestrae within the vascular walls and increased extravasation of nucleic acids. Delivery to organs other than the liver, including the kidneys, has also been reported, albeit at reduced efficiency [84]. The hydraulic pressure in the glomerular capillary is estimated at 45–70 mmHg, which is higher than that of the peripheral capillary or portal venules. Further increases in venous and capillary pressure produce temporary disruption of glomerular vessel beds and exposure of the tubular epithelium to siRNA.

Despite initial success in rodent models, the traumatic nature of hydrodynamic delivery and the possibility of liver damage preclude its application in humans. Among possible alternatives are deliveries through the ureter and into surgically accessed iliac or renal arteries or veins. Access through the renal artery allows targeting at the tubular epithelium [64, 70], while injection into local veins is aimed at both tubules and the interstitium [44]. It is important to note that injection into the renal artery implies temporary occlusion and exposure to mild ischemia, which may provide ischemic preconditioning of the organ and decrease its sensitivity to subsequent ischemic insult [85]. This needs to be taken into consideration in designing experiments in model animals.

The efficiency of siRNA delivery in vivo may be improved by electroporation, as has been shown in a rat model of glomerulonephritis [64, 70]. However, it was shown that electroporation may induce stress signaling [47]. In this light, sonoporation was suggested as an alternative [86]. Some researchers have also suggested the possibility of direct injections of siRNA into the renal parenchyma. It is unlikely that this approach will gain traction in clinical use, because of its invasiveness and the risk of infection [83].

6 Targets for siRNA-Based Interventions in Kidney Transplantation

To our knowledge, no systematic screens for siRNA targets most relevant to kidney transplantation have been attempted so far. A majority of researchers have arrived at one or another target of interest by analyzing current literature that dissects the pathological processes described above. The key molecular players that underpin the physiology of ischemia–reperfusion are well studied across a variety of organs and systems. In fact, none of the commonly studied molecules are specific to the processes within the kidney grafts. However, most biological networks show modular properties [87]. In a practical sense that is relevant to the topic of this review, this means that if anti-TGF-β siRNA is shown to successfully suppress collagen deposition in the liver, it will most likely demonstrate the same properties in the lung model. Similarly, if giving siRNA-based therapeutics suppressed rejection of, say, the heart, it is likely to be successful as an anti-rejection treatment for kidney grafts.

We have compiled a list of 53 siRNA targets described in the literature, as tested in in vivo or in vitro experiments aimed at the testing of siRNA efficiency with an eventual goal to develop molecular drugs for various transplantation-related applications (Table 3). This list has been associated with GenBank gene accession numbers and further analyzed by MetaCore™ analysis software (GeneGo, Inc., St. Joseph, MI, USA) to sort constituent genes into cellular pathways and disease-specific pathogenetic networks. An assessment of statistical significance

Table 3 List of small interfering RNA (siRNA) targets tested in models relevant to kidney grafting

Gene symbol and GenBank accession number	Gene name	Gene function
Apoptosis		
AIFM1 (NM_004208.3)	Mitochondrion-associated apoptosis-inducing factor 1	During apoptosis, AIFM1 translocates from the mitochondria to the nucleus to function as a pro-apoptotic factor in a caspase-independent pathway, while in normal mitochondria, it functions as an anti-apoptotic factor via its oxidoreductase activity
APAF1 (NM_181861.1)	Apoptotic peptidase activating factor 1	Oligomeric Apaf-1 mediates cytochrome C-dependent autocatalytic activation of pro-CASP9, leading to activation of CASP3 and apoptosis
BAD (NM_004322.3)	BCL2-associated agonist of cell death	Promotes cell death
BAX (NM_004324.3)	BCL2-associated X protein	Promotes activation of CASP3 and thereby apoptosis
CASP12 (NM_001191016.1)	Caspase 12	In rodents, mediates apoptosis in response to endoplasmic reticulum stress; in some humans, this gene has a premature stop codon
CASP3 (NM_004346.3)	Caspase 3, apoptosis-related cysteine peptidase	Involved in the activation cascade of caspases responsible for apoptosis execution
CASP7 (NM_001227.4)	Caspase 7, apoptosis-related cysteine peptidase	Involved in the activation cascade of caspases responsible for apoptosis execution
CASP8 (NM_001080124.1)	Caspase 8, apoptosis-related cysteine peptidase	Cleaves and activates CASP3, CASP4, CASP6, CASP7, CASP9, and CASP10
DFFB (NM_004402.2)	DNA fragmentation factor, 40kda, beta polypeptide (caspase-activated DNAse)	Substrate for CASP3; triggers DNA fragmentation during apoptosis
FAS (NM_000043.4)	Fas cell surface death receptor	Receptor for TNFSF6/FASLG; activates caspase 8, which, in turn, initiates the subsequent cascade of caspases
GADD45B (NM_015675.3)	Growth arrest and DNA-damage-inducible, beta	Modulates signaling in response to physiological and environmental stressors, which results in either cell cycle arrest, DNA repair, cell survival and senescence, or apoptosis
Pro-inflammatory cytokines and related signaling		
TNF (NM_000594.3)	Tumor necrosis factor	Multifunctional pro-inflammatory cytokine; belongs to the TNF superfamily
TNFRSF1A (NM_001065.3)	Tumor necrosis factor receptor superfamily, member 1A	Major receptors for TNF-α; can activate NF-κB, mediate apoptosis, and function as a regulator of inflammation
RIPK1 (NM_003804.3)	Receptor (TNFRSF)-interacting serine-threonine kinase 1	Transduces inflammatory and cell-death signals (programmed necrosis) following death receptor ligation, activation of pathogen recognition receptors, and DNA damage
IL1B (NM_000576.2)	Interleukin 1, beta	Important mediator of the inflammatory response
Complement components and receptors		
C3 (NM_000064.2)	Complement component 3	Central role in activation of both classical and alternative complement system
C5 (NM_001735.2)	Complement component 5	Fifth component of complement, which plays an important role in inflammatory and cell-killing processes
C5AR1 (NM_001736.3)	Complement component 5a receptor 1	Receptor for the chemotactic and inflammatory peptide anaphylatoxin c5a; stimulates chemotaxis, granule enzyme release, and superoxide anion production
Cytoplasmic signal transduction components		
ABL1 (NM_007313.2)	C-abl oncogene 1, non-receptor tyrosine kinase	Protein tyrosine kinase; implicated in processes of cell differentiation, cell division, cell adhesion, and stress response
MAPK14 (NM_001315.2)	Mitogen-activated protein kinase 14	Serine/threonine kinase; acts as an essential component of the MAP kinase signal transduction pathway
PTPN1 (NM_002827.2)	Protein tyrosine phosphatase, non-receptor type 1	Protein tyrosine phosphatase
Non-caspase proteases		
CAPN1 (NM_001198868.1)	Calpain 1, (mu/I) large subunit	Non-lysosomal, intracellular cysteine protease; catalyzes limited proteolysis of substrates involved in cytoskeletal remodeling and signal transduction

Table 3 continued

Gene symbol and GenBank accession number	Gene name	Gene function
MMP9 (NM_004994.2)	Matrix metallopeptidase 9 (gelatinase B, 92kda gelatinase, 92kda type IV collagenase)	Breakdown of extracellular matrix
CYLD (NM_015247.2)	Cylindromatosis (turban tumor syndrome)	Functions as a deubiquitinating enzyme
Cytoplasmic enzymes		
NOX4 (NM_016931.3)	NADPH oxidase 4	Acts as an oxygen sensor and catalyzes reduction of molecular oxygen to various ROS
OGT (NM_181672.2)	O-linked *N*-acetylglucosamine (glcnac) transferase	Glycosyltransferase; catalyzes addition of a single *N*-acetylglucosamine in O-glycosidic linkage to serine or threonine residues
P4HA2 (NM_001017973.1)	Prolyl 4-hydroxylase, alpha polypeptide II	Component of prolyl 4-hydroxylase; key enzyme in collagen synthesis
PPIF (NM_005729.3)	Peptidylprolyl isomerase F	Catalyzes cis-trans isomerization of proline imidic peptide bonds in oligopeptides and accelerates folding of proteins
SMPD1 (NM_000543.4)	Sphingomyelin phosphodiesterase 1, acid lysosomal	Converts sphingomyelin to ceramide; also has phospholipase C activities toward 1,2-diacylglycerolphosphocholine and 1,2-diacylglycerolphosphoglycerol
Channels and carriers		
RYR2 (NM_001035.2)	Ryanodine receptor 2 (cardiac)	Component of a calcium channel in cardiac muscle sarcoplasmic reticulum
SLC25A4 (NM_001151.3)	Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4	Catalyzes the exchange of cytoplasmic with mitochondrial ATP across the mitochondrial inner membrane
SLC8A1 (NM_021097.2)	Solute carrier family 8 (sodium/calcium exchanger), member 1	Rapidly transports Ca^{2+} during excitation–contraction coupling; Ca^{2+} is extruded from the cell during relaxation so as to prevent overloading of intracellular stores
ATP6V1G2 (NM_130463.3)	Atpase, H+ transporting, lysosomal 13kda, V1 subunit G2	Catalytic subunit of the peripheral V1 complex of V-atpase, which is responsible for acidifying a variety of intracellular compartments in eukaryotic cells
Toll-like receptors		
TLR2 (NM_003264.3)	Toll-like receptor 2	Plays a fundamental role in pathogen recognition and activation of innate immunity
TLR4 (NM_138554.4)	Toll-like receptor 4	Plays a fundamental role in pathogen recognition and activation of innate immunity
Transcriptional regulation		
HDAC1 (NM_004964.2)	Histone deacetylase 1	Histone acetylation and deacetylation, thus regulating eukaryotic gene expression
HIF1A (NM_001530.3)	Hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	Master regulator of cellular and systemic homeostatic response to hypoxia by activating transcription of many genes, including those involved in energy metabolism, angiogenesis, and apoptosis
PAWR (NM_002583.2)	PRKC, apoptosis, WT1, regulator	Represses and activates transcription
SMARCA4 (NM_001128844.1)	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4	Required for transcriptional activation of genes normally repressed by chromatin
RELB (NM_006509.3)	V-rel avian reticuloendotheliosis viral oncogene homolog B	Component of pleiotropic transcription factor NF-κB
TP53 (NM_000546.5)	Tumor protein p53	Tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains
Secreted molecules other than pro-inflammatory cytokines		
ADIPOQ (NM_001177800.1)	Adiponectin, C1Q and collagen domain containing	Important adipokine involved in control of fat metabolism and insulin sensitivity, with direct anti-diabetic, anti-atherogenic, and anti-inflammatory activities

Table 3 continued

Gene symbol and GenBank accession number	Gene name	Gene function
AZGP1 (NM_001185.3)	Alpha-2-glycoprotein 1, zinc-binding	Stimulates lipid degradation in adipocytes and causes extensive fat losses
WNT3A (NM_033131.3)	Wingless-type MMTV integration site family, member 3A	Implicated in oncogenesis and in several developmental processes, including regulation of cell fate and patterning during embryogenesis
Miscellaneous		
CAV1 (NM_001753.4)	Caveolin 1, caveolae protein, 22 kDa	Scaffolding protein and main component of the caveolae plasma membranes found in most cells
DNM1L (NM_012062.3)	Dynamin 1-like	Functions in mitochondrial and peroxisomal division
DNM2 (NM_001005360.2)	Dynamin 2	Microtubule-associated force-producing protein involved in producing microtubule bundles and able to bind and hydrolyze GTP
HSP90B1 (NM_003299.2)	Heat shock protein 90 kDa beta (Grp94), member 1	Molecular chaperone; functions in processing and transport of secreted proteins
NUDT13 (NM_015901.4)	Nudix (nucleoside diphosphate linked moiety X)-type motif 13	Not clear
TNFAIP8L1 (NM_001167942.1)	Tumor necrosis factor, alpha-induced protein 8-like 1	Not clear
SPATA2 (NM_001135773.1)	Spermatogenesis associated 2	Potential role in spermatogenesis and pancreatic β cell function
SYCP2 (NM_014258.2)	Synaptonemal complex protein 2	Major component of the synaptonemal complex; may bind DNA at scaffold attachment regions

ATP adenosine triphosphate, *GTP* guanosine triphosphate, *MAP* mitogen-activated protein, *NF* nuclear factor, *ROS* reactive oxygen species, *TNF* tumor necrosis factor, *V-atpase* vacuolar atpase

throughout MetaCore™ is based on the *P* value, which is calculated on the basis of hypergeometric distribution. Interestingly, the majority of the genes already tested in transplantation-related models belong to either the apoptosis- or immune rejection-centered networks. The top-rated pathways and networks included "Apoptosis and survival, TNF-alpha-induced Caspase-8 signaling", "Apoptosis and survival, Caspase cascade", "Immune response, Alternative complement

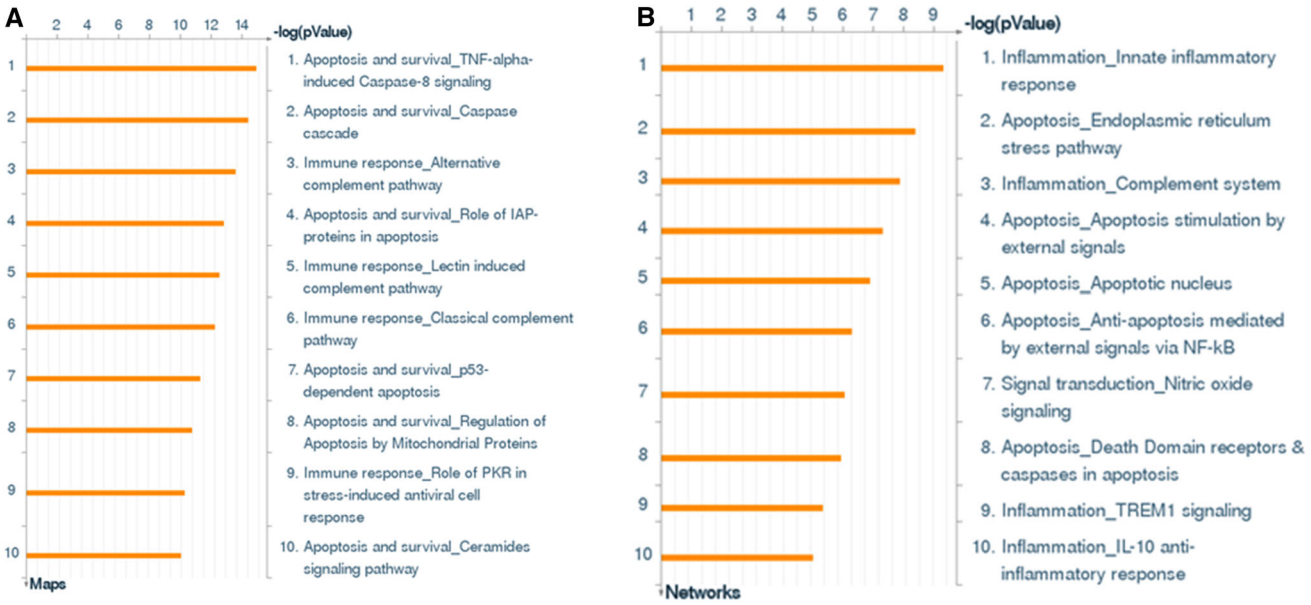

Fig. 4 Results of MetaCore™ analysis with respective significance scores: **a** top pathway maps; **b** top process networks. *IAP* inhibitor of apoptosis, *IL* interleukin, *NF* nuclear factor, *PKR* protein kinase R, *TNF* tumor necrosis factor, *TREM1* triggering receptor expressed on myeloid cells 1

pathway", "Apoptosis and survival, Role of IAP-proteins in apoptosis" and "Immune response, Lectin induced complement pathway" (Fig. 4). Among the top 5 Gene Ontology (GO) biological processes defined as operations or sets of molecular events with a defined beginning and end, pertinent to the functioning of cells, tissues, and organs, four were related to apoptosis, and one described a response to hypoxia. Importantly, the lists of the apoptosis-related and complement cascade-related molecules commonly targeted by siRNA do not intersect, and so are the respective pathway maps. This observation indicates that there is an opportunity for therapeutic siRNA combinations that may be delivered within the same delivery vector or injected at the same time and, by targeting more than one pathway, or by hitting the same pathways within two different key points, will augment the effects of each other.

Surprisingly, the list of Metabolic Networks provided some additional insights, with statistically significant highlighting of sphyngomyelin ($p < 7e^{-4}$), D-glucoronic acid ($p < 4e^{-3}$), ceramide ($p < 4e^{-3}$), sucrose ($p < 7e^{-3}$), and others. These networks should be expected to be at least to some degree perturbed by one or another siRNA intervention tested for their application in kidney transplantation.

7 Conclusion

To curtail the damage to the transplanted kidney that is caused by ischemia–reperfusion injury and the recipient's immune system, siRNA technology is being explored. To date, most attempts at siRNA-based therapy for transplantation-related conditions have remained at the in vitro stage, with only a few of them being advanced into animal models. Hydrodynamic intravenous injection of naked or carrier-bound siRNAs is currently the most common route for delivery of therapeutic constructs. To our knowledge, no systematic screens for siRNA targets most relevant to kidney transplantation have been attempted so far. Typically, researchers have arrived at one or another target of interest by analyzing current literature that dissects pathological processes taking place in transplanted organs. A majority of the genes that make up the list of 53 siRNA targets that have been tested in transplantation-related models so far belong to either the apoptosis- or immune rejection-centered networks. There is an opportunity for therapeutic siRNA combinations that may be delivered within the same delivery vector or injected at the same time and, by targeting more than one pathway, or by hitting the same pathways within two different key points, will augment the effects of each other.

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