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REVIEW ARTICLE

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Transcription factor decoy oligonucleotide-based therapeutic strategy for renal disease

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Abstract
Renal disease, including slight renal injuries, has come to be seen as one of the risk factors for cardiovascular events. At present, most conventional therapy is inefficient, and tends to treat the symptoms rather than the underlying causes of the disorder. Gene therapy based on oligonucleotides (ODN) offers a novel approach for the prevention and treatment of renal diseases. Gene transfer into somatic cells to interfere with the pathogenesis contributing to renal disease may provide such an approach, leading to the better prevention and treatment of renal disease. The major development of gene transfer methods has made an important contribution to an intense investigation of the potential of gene therapy in renal diseases. Amazing advances in molecular biology have provided the dramatic improvement in the technology that is necessary to transfer target genes into somatic cells. Gene transfer methods, especially when mediated by several viral vectors, have improved to a surprising extent. In fact, some (retroviral vectors, adenoviral vectors, or liposome-based vectors, etc.) have already been used in clinical trials. On the other hand, recent progress in molecular biology has provided new techniques to inhibit target gene expression. The transfer of *cis*-element double-stranded ODN (= decoy) has been reported to be a powerful novel tool in a new class of antigene strategies for gene therapy. The transfer of decoy ODN corresponding to the *cis* sequence will result in attenuation of the authentic *cis–trans* interaction, leading to the removal of *trans*-factors from the endogenous *cis*-elements with a subsequent modulation of gene expression.

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Introduction

As a consequence of developments in the field of molecular biology and their impact on our understanding of the mechanisms of disease processes, therapeutic approaches that exploit our expanding knowledge of the structure and function of molecules are being developed. A major focus of cellular and molecular research has been to develop a strategy to regulate gene expressions in an effort to treat and cure a variety of diseases and abnormal physiological conditions. The technologies of oligodeoxynucleotides (ODN) have received considerable attention because they provide a rational way to design sequence-specific ligands of nucleic acids or DNA-binding regulatory proteins as a tool for the selective regulation of a specific gene expression. This is of particular interest for the development of new pharmacological interventions to treat diseases characterized by aberrant activation and expression of genes whose products are involved in the initiation and progression of pathogenesis. In particular, as altered activation of transcription factors has become a better understood component of many pathways of disease pathogenesis, including cancer, viral infection, and chronic inflammatory diseases, the development of molecular strategies targeting transcription activating proteins has emerged as an attractive field of investigation.[1] One of the most successful ODN-based approaches has been the use of a synthetic double-strand ODN (= decoy) containing an enhancer element.[2–10] Nucleic acid molecules with a high affinity for a target transcription factor can be introduced into cells as a decoy *cis*-element to bind to a transcription factor and alter gene expressions. After delivery into the nucleus of the target cell, decoy ODN can bind to free transcription factors and block the interaction of these factors to the promoter region of target genes. Alternatively, decoy ODN against a negative transcription factor

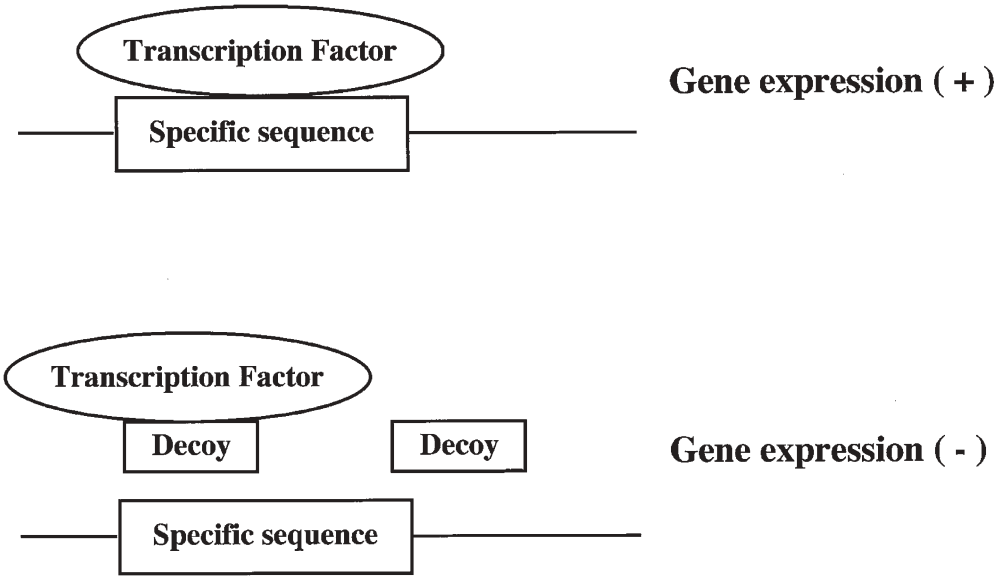

Fig. 1. Scheme of the decoy strategy. In the basal state, transcription factor is bound to *cis*-element, resulting in the continuous activation of target gene expression. Decoy ODN binds to transcription factor, resulting in the prevention of transcription factor interaction and transactivation of transcription factor-promoting target gene expression

may act to enhance expressions of otherwise suppressed genes. Decoy ODN have been used successfully in vitro and, more importantly, cells in vivo in intact animal models.[11–21] Decoy ODN have been used in variety of forms, ranging from short 10–20 base pairs (bp) ODN to plasmid DNA containing multiple repeats of the consensus sequence.[22] To enhance the efficacy and duration of activity of decoy ODN, phosphorothioate ODN that are resistant to nuclease activity have often been used. Modified ODNs accumulate in cells more effectively than standard ODN, and modulate gene expressions in a specific manner. The decoy ODN approach may enable us to treat diseases by the modulation of endogenous transcriptional regulation of target genes. Several reports have described applications of the decoy ODN approach as in vivo gene therapy.[11–21] Previously, many researchers used antisense technology as a loss-of-function approach at the transcriptional and translational levels. However, the precise mechanisms of this technology are still unclear. On the other hand, the mechanism of the decoy ODN approach is very simple, and is also applicable as a loss-of-function approach at the pretranscriptional level. This approach seems to be particularly attractive for several reasons: (1) potential targets are plentiful and readily identifiable; (2) synthesis of decoy ODN is relatively simple and can be targeted to specific issues; (3) knowledge of the exact molecular structure of target transcription factors is not necessary; (4) decoy ODN may be more effective than antisense ODN in terms of inhibiting target gene expressions.

In this review, we focus on the application of the decoy ODN approach to renal diseases by demonstrating successful results from several animal models.

Principles of decoy strategy

The correct regulation of gene expression is essential for both normal development and the correct functioning of the adult organism. Such regulation is usually achieved at the level of DNA transcription, a process that controls which genes are transcribed into RNA by the enzyme RNA polymerase, although posttranscriptional regulation is also important.[23] The transcription of a specific gene is controlled by a regulatory protein known as a transcription factor.[23] Transcription factors have been grouped into families on the basis of shared DNA-binding motifs. Other regions of the factors interact with RNA polymerase and its associated proteins to increase or decrease the rate of transcription. The vital role of these factors, together with the fact that a single factor can affect the expression of many genes, suggests that the inactivation of a transcription factor as a result of an inherited mutation is incompatible with survival. Several years ago, we hypothesized that synthetic double-stranded ODN with a high affinity for a transcription factor may be introduced in vivo as a decoy *cis*-element to bind to a transcription factor and block the activation of genes mediating such diseases, resulting in an effective therapy for treating the diseases, as the transfer of decoy ODN corresponding to the *cis* sequence will result in attenuation of the removal of *trans*-factors from the endogenous *cis*-element, with subsequent modulation of gene expression (Fig. 1).[24–29] Although the mechanisms of antisense ODN are still unclear, the principle of the transcription factor decoy approach is simply the reduction of promoter activity due to the inhibition of the binding of a transcription factor to specific sequences in the promoter region. Alternatively, this strategy also provides a powerful tool to study endogenous gene regulation in vivo as well as in vitro by modulation of endogenous transcriptional regulation.

Gene and ODN transfer methods into the kidney

Although cells have the capacity to take up naked DNA, this process is extremely inefficient and results in transient transgene expression. This is no surprise given the number

of steps encountered that could potentially limit the efficiency of gene transfer. Therefore, a number of viral and nonviral vectors have been developed to improve the efficiency of gene transfer.[30] Viral vectors are replication-defective viral particles that retain the ability to enter target cells and transfer their genetic material into the cell's genome. Replacing genes required for viral replication with an expression cassette for the therapeutic gene transforms viruses into safe vectors that provide delivery and expression of the transgene within the host cell.

An ideal vector efficiently introduces recombinant genes into many cell types and results in long-term and stable expression of the gene. Unfortunately, no ideal vector exists at present. Instead, a number of viral and nonviral vectors have been developed, each with unique properties that will be useful for specific indications (Table 1). It is important to note that the science of vectors is a field in itself, with many research laboratories devoted to optimizing vector construction and delivery. There are several parameters that must be taken into consideration when evaluating a vector, including its efficiency in transducing different cell types in vitro and in vivo, the size and type of genetic material that it can introduce, its ability to target its genetic material to specific cell compartments, the intracellular stability of the genetic material, its ability to produce stable transcription and translation of the transgenes, a lack of immunity against the transgene in vivo, and a lack of cytotoxicity.

We have developed a gene transfer vector by combining the hemagglutinating virus of Japan (HVJ or Sendai virus) with liposome-based nonviral vectors.[31–35] Liposomal vectors are taken up into cells by phagocytosis, and their contents are easily degraded in the lysosome before reaching the cytoplasm. However, if liposomes could be made to fuse with the plasma membrane, their contents could be introduced directly into the cytoplasm, avoiding degradation by the endocytic pathway. HVJ can fuse with cell membranes and also with liposomes.[29] Two distinct glycoproteins are required for cell fusion: HN is required for the binding of HVJ to cell-surface sialic-acid receptors, and F binds to lipids, such as cholesterol, in the cell membrane to induce cell fusion. Using the HVJ envelope, we developed a novel hybrid-type liposome called the HVJ-liposome.[29] The liposome is fused with UV-inactivated HVJ to form the HVJ-liposome, which can fuse with the cell surface to deliver plasmid DNA directly to the cytoplasm (Fig. 2). Utilizing this gene transfer method, we developed an efficient method of gene transfer into the vessel wall, the myocardium, or the kidney, as reported previously.[26,32,36] Importantly, moreover, it was revealed that this method enhances the transfer efficiency of antisense ODN or decoy ODN in vivo.[27,28,36–38]

Recently, several studies have shown that ultrasound, used either alone or in combination with ultrasound contrast agents, can increase cell membrane permeability to macromolecules such as plasmid DNA.[39–43] This phenomenon has been referred to as sonoporation.[40] Most sonoporation studies have been carried out on cultured cells,[39–44] tumors in vivo,[45–48] or skeletal muscle.[49–51] Moreover, we have recently found that this approach is applicable to gene transfer into the artery.[52] However, to our knowledge, there has been no previous investigation of whether ultrasound is able to enhance plasmid DNA transfer into the kidney. We have also found that ultrasound and ultrasound contrast reagents, microbubbles, can transfer both plasmid DNA and decoy ODN efficiently.[53–55] The use of ultrasound as an adjuvant measure to enhance plasmid DNA and decoy ODN delivery has a number of advantageous features, which should increase the overall prospects for therapeutic applications of plasmid DNA and decoy ODN in the kidney. It is true that electroporation-mediated transfer resulted in rather high gene expression.[56–58] However, in contrast to electroporation, ultrasound is a nonpainful and well-established tool in clinical medicine. The noninvasive nature of ultrasound and the absence of neutralizing antibodies against plasmid DNA also raise the possibility that treatment could easily be repeated on a relatively frequent basis.

Table 1. Vectors for human gene therapy. This table summarizes the characteristics of each vector

	Advantages	Disadvantages
Adenoviruses	Highly efficient	Inflammation
Adeno-associated Viruses	Integration (?)	Difficult to concentrate DNA < 5 Kb
Retroviruses	Integration Long-term expression	Difficult to concentrate
Liposomes	Simple to produce Safety profile	Inefficient
Plasmid DNA	Very simple to produce Safety profile	Very inefficient

Applications of decoy strategy to renal diseases

Mesangial cell proliferating glomerulonephritis

Mesangial cell (MC) cycle progression is believed to play a pivotal role in the pathogenesis of human glomerular diseases such as membranoproliferative glomerulonephritis, lupus nephritis, IgA nephropathy, and focal glomerulosclerosis.[59,60] When accompanied by abnormalities in matrix turnover, this acute glomerulonephritis may culminate in chronic glomerular sclerosis, causing renal failure.[59,60] The factors responsible for abnormal MC proliferation and matrix deposition have not been fully identified, but previous studies have documented the importance of such growth factors as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), insulin-like growth factor-I (IGF-I), and basic fibroblast growth factor (bFGF) in the pathogenesis of glomerular lesion formation.[61–64] Since growth factors mediate proliferation by activation of the final common pathway of cell-cycle progression, we hypothesized that a more potent abrogation of abnormal proliferation could be achieved by blockade of cell-cycle regulatory genes. In fact, sequential activation of such critical cell-cycle regulatory genes as *c-myb*, *c-myc*, cdc2 kinase,

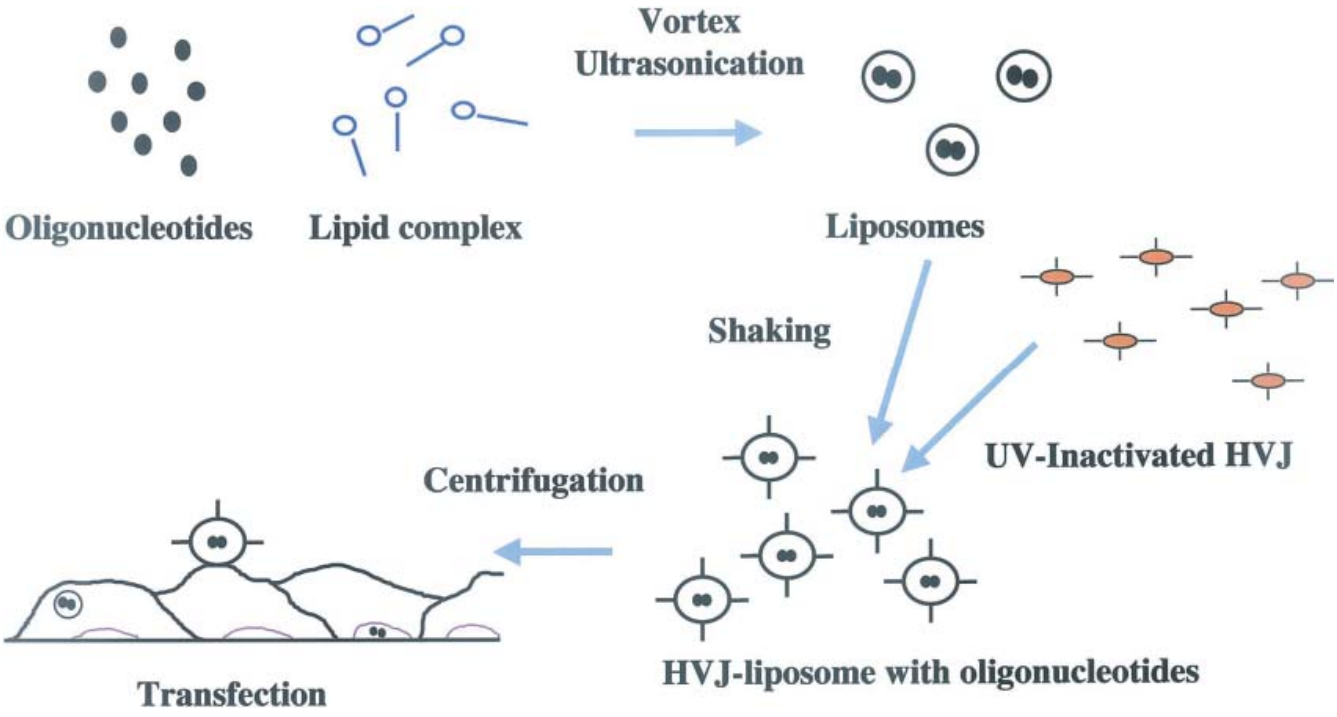

Fig. 2. Procedure for preparing hemagglutinating virus of Japan (*HVJ*)-liposome. First, oligonucleotides (ODN) were encapsulated into liposomes. These liposomes were treated with HVJ inactivated by ultraviolet irradiation to form HVJ-liposome. This suspension was directly injected in vivo into target organs

cdk2 kinase, and proliferating-cell nuclear antigen (PCNA) is under the control of the transcription factor, E2F.[65–67] We therefore proposed to block E2F-mediated genetic programs to halt the progress of MC through the cell cycle. We hypothesized that modulating E2F transcriptional activation via decoy ODN would inhibit the MC cycle progression and subsequent lesion formation in experimental glomerulonephritis, because E2F-mediated regulation is postulated to be a ubiquitous mechanism of mammalian proliferation.

We examined the distribution of fluorescein isothiocyanate (FITC)-labeled double-stranded E2F decoy ODN transferred directly into the left renal artery of normal rats in vivo. The FITC-labeled E2F decoy ODN was packaged in liposomes with or without HVJ in their outer coat. ODN packaged in HVJ-liposomes could be detected by direct immunofluorescence in glomerular cells and tubular epithelial cells 24 h after intrarenal artery transfer (Fig. 3). In contrast, naked FITC-labeled E2F decoy ODN, ODN packaged in liposomes without HVJ, or ODN packaged in Lipofectamine localized predominantly in tubular epithelial cells. Approximately 30% of the glomeruli in HVJ-liposomes-treated kidneys were labeled. Glomerular uptake was greatest 24 h after transfer. FITC glomerular labeling gradually decreased on days 2, 5, and 7 (data not shown). However, even on day 7 after transfer, FITC staining could be seen in some glomeruli. We then examined whether or not E2F inhibition in vivo would result in a decrease of the down-stream cell-cycle regulatory genes, PCNA, and adk2 kinase. On day 2 after the injection of anti-Thy 1 antiserum, glomerular mRNA levels of these genes were increased, as assessed by reverse transcription–polymerase chain reaction (RT–PCR) (Fig. 4). The transfer of E2F decoy ODN in vivo was associated with decreased PCNA and cdk2 kinase mRNA expression, whereas missense (MIS) decoy ODN treatment did not affect the level of mRNA expression of these genes.

A histological examination of kidneys at day 5 in anti-Thy 1-treated rats revealed diffuse glomerular injury characterized by an increase in MC number and mesangial matrix expansion (Fig. 5). In comparison, an overall reduction in cellularity and matrix expansion was seen in E2F decoy ODN-treated left kidneys as compared with right kidneys (Fig. 5). This histological improvement was not observed in MIS decoy ODN-treated left kidneys (Fig. 5). The overall degree of glomerular histological injury was reduced from 32% to 19% of all glomeruli in right vs. left kidneys of E2F decoy ODN-treated rats ($P < 0.01$).

Glomerular diseases are important causes of human chronic renal failure. MC proliferation and abnormal matrix deposition are key features of progressive glomerular injury. MC proliferation in the anti-Thy 1 model is dependent on growth factor and cytokine activation of cell-cycle progression.[68] The transcription factor E2F has been implicated in cell-cycle control through its complex association with the retinoblastoma (RB) tumor suppressor protein, cyclin A, and cdks.[65,67,69,70] The inactivation of RB by phosphorylation results in the release of E2F from the complex. The subsequent binding of E2F to its corresponding *cis*

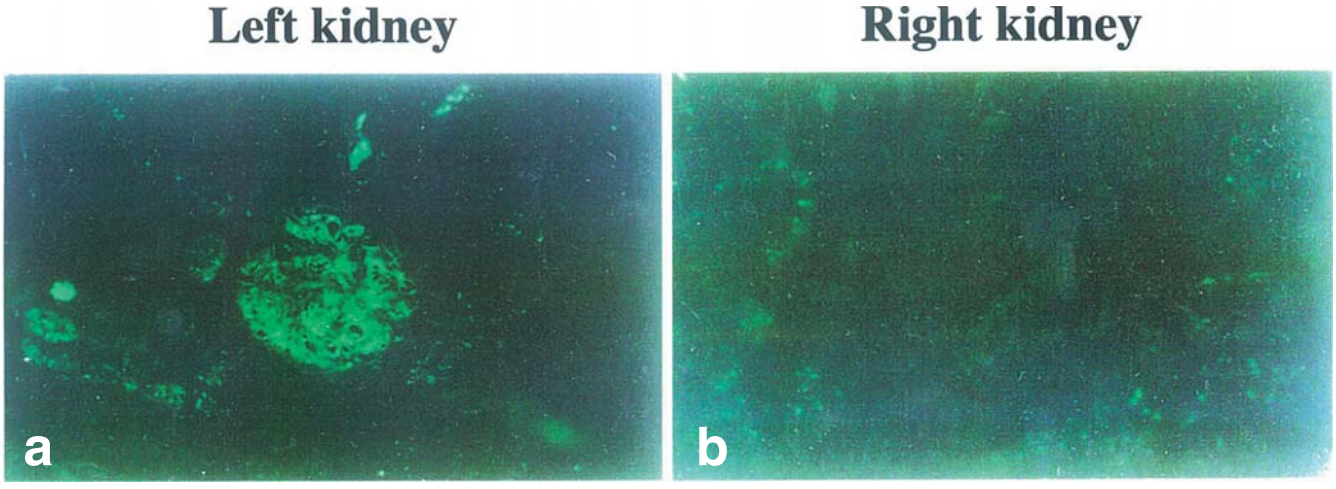

Fig. 3. Distribution of transferred ODN. Twenty-four hours after the intrarenal transfer of fluorescein isothiocyanate (FITC)-labeled E2F decoy ODN (15 μM), rats were killed for tissue analysis by immunofluorescence microscopy. The marker is clearly seen in glomeruli and in tubular epithelium of the kidney transferred with HVJ-liposome solution (*left*), but not in the control kidney (*right*)

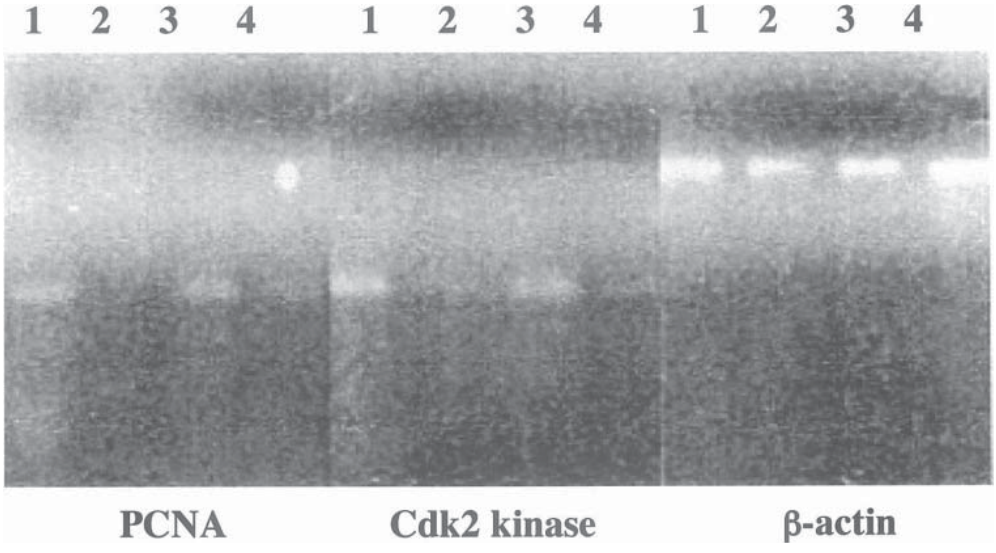

Fig. 4. Proliferating-cell nuclear antigen (*PCNA*) and cdk2 kinase gene expression assessed by reverse transcriptase–polymerase chain reaction (RT–PCR). Two days after transfer of E2F and missense (MIS) decoy ODN, glomerular total RNA was extracted and subjected to RT–PCR for the detection of PCNA, cdk2 kinase, and β-actin. E2F decoy ODN treatment was associated with a reduction in PCNA and cdk2 kinase RNA. *Lane 1*, anti-Thy 1-treated rat glomerular total RNA; *Lane 2*, untreated rat glomerular total RNA; *Lane 3*, anti-Thy 1 and MIS decoy ODN-treated rat glomerular total RNA; *Lane 4*, anti-Thy 1 and E2F decoy ODN-treated rat glomerular total RNA

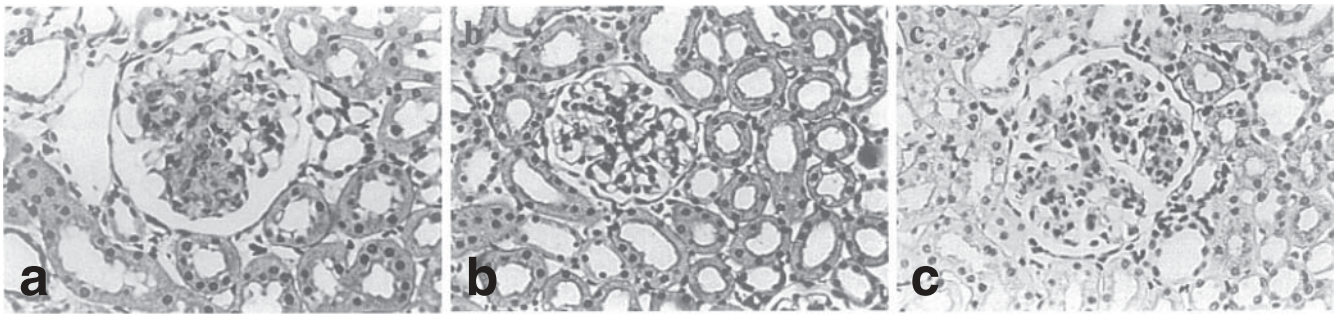

Fig. 5. Glomerular histological changes after decoy ODN transfer. PAS-stained renal sections from left kidneys exposed to anti-Thy 1 antiserum and treated with no decoy ODN transfer (**a**), E2F decoy ODN (**b**), and MIS decoy ODN (**c**). Diffuse glomerular injury was seen in anti-Thy 1-treated rats (**a**), and was also present in MIS decoy ODN-treated left kidneys (**c**). Histologically normal glomeruli were seen only in E2F decoy ODN-treated left kidneys (**b**)

element results in the coordinated activation of cell-cycle regulatory genes, including *c-myb*, *c-myc*, PCNA, and cdk2 kinase,[71–74] and cell proliferation ensues. The cellular transcription factor E2F has been shown to be important in cell proliferation control.[71–74] We show a potential strategy for gene therapy for proliferative glomerular diseases using E2F decoy ODN to inhibit cell-cycle progression.

Inflammatory glomerulonephritis

Crescentic glomerulonephritis is a rapidly progressive form of kidney disease with a poor prognosis. The aggressive nature of this disease is attributed to the prominent leukocytic infiltration observed in biopsy samples.[75,76] This infiltrate is associated with marked up-regulation of renal expression of proinflammatory cytokines (interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α)), chemokines (monocyte chemoattractant protein-1 (MCP-1)), and leukocyte adhesion molecules (intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)).[77–80] Experimental models of crescentic glomerulonephritis, such as antiglomerular basement membrane (anti-GBM) disease, have been shown to be leukocyte-dependent.[81] The transcription factor nuclear factor-kappa B (NF-κB) is important in the coordinated expression of various proinflammatory molecules.[82,83] Therefore, we postulated that inhibiting the action of NF-κB using a decoy ODN could block the underlying inflammatory response in crescentic glomerulonephritis.

Rats transferred with scrambled (SD) decoy ODN developed crescentic glomerulonephritis in terms of severe renal injury, as demonstrated by heavy urinary protein excretion (Fig. 6), and marked histological damage, as shown by the presence of glomerular hypercellularity, focal glomerular sclerosis, glomerular crescent formation, and interstitial lesions (Fig. 7). In contrast, transfer with NF-κB decoy ODN caused a reduction of approximately 50% in urinary protein excretion over a 7-day period. There was also a substantial reduction in histological damage in the NF-κB decoy ODN-treated kidney (Fig. 7). Northern blot analysis showed that expression of IL-1β, TNF-α, and ICAM-1 is increased in SD decoy ODN-treated anti-GBM disease compared with normal kidney, and reduced by treatment with NF-κB decoy ODN. A more detailed analysis of IL-1β and TNF-α expression was performed by in situ hybridization. Constitutive expression of IL-1β mRNA in normal kidney was restricted to a small number of glomerular cells and a minority of tubules (Fig. 8). There was a dramatic increase in the number of glomerular cells expressing IL-1β in SD decoy ODN-treated anti-GBM disease, and most tubules showed IL-1β mRNA expression (Fig. 8). Treatment with NF-κB decoy ODN caused a substantial reduction in both glomerular and tubular IL-1β

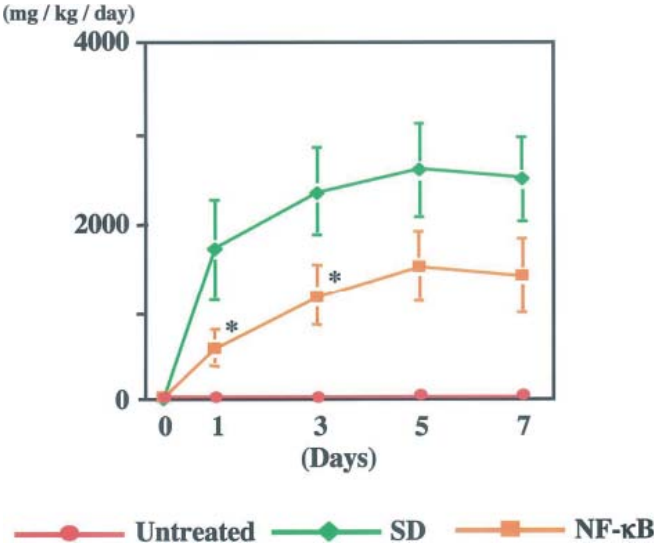

Fig. 6. Inhibition of renal injury in anti-glomerular basement membrane (GBM) disease by NF-κB decoy ODN. Renal injury was quantitated by the amount of protein excreted in a 24-h urine collection (mg/kg/24 h). *Squares*, SD decoy ODN-treated rats; *circles*, NF-κB decoy ODN-treated rats. Data are shown as mean ± SEM. $*P < 0.01$ vs. SD decoy ODN-treated rats by ANOVA

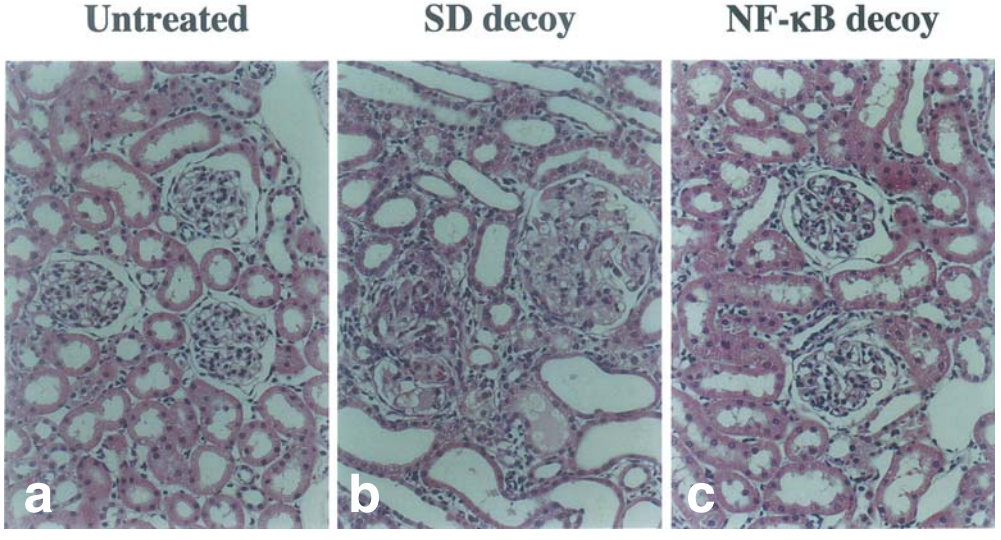

Fig. 7. Inhibition of histological damage in anti-GBM disease by NF-κB decoy ODN. Compared to normal rat kidney (**a**), the induction of anti-GBM disease in SD decoy ODN-treated rats caused marked histological damage, including glomerular hypercellularity, crescent formation, tubular atrophy, and interstitial fibrosis (**b**). **c** Treatment of anti-GBM disease with NF-κB decoy ODN caused a significant reduction in the severity of histological damage compared with SD decoy ODN treatment

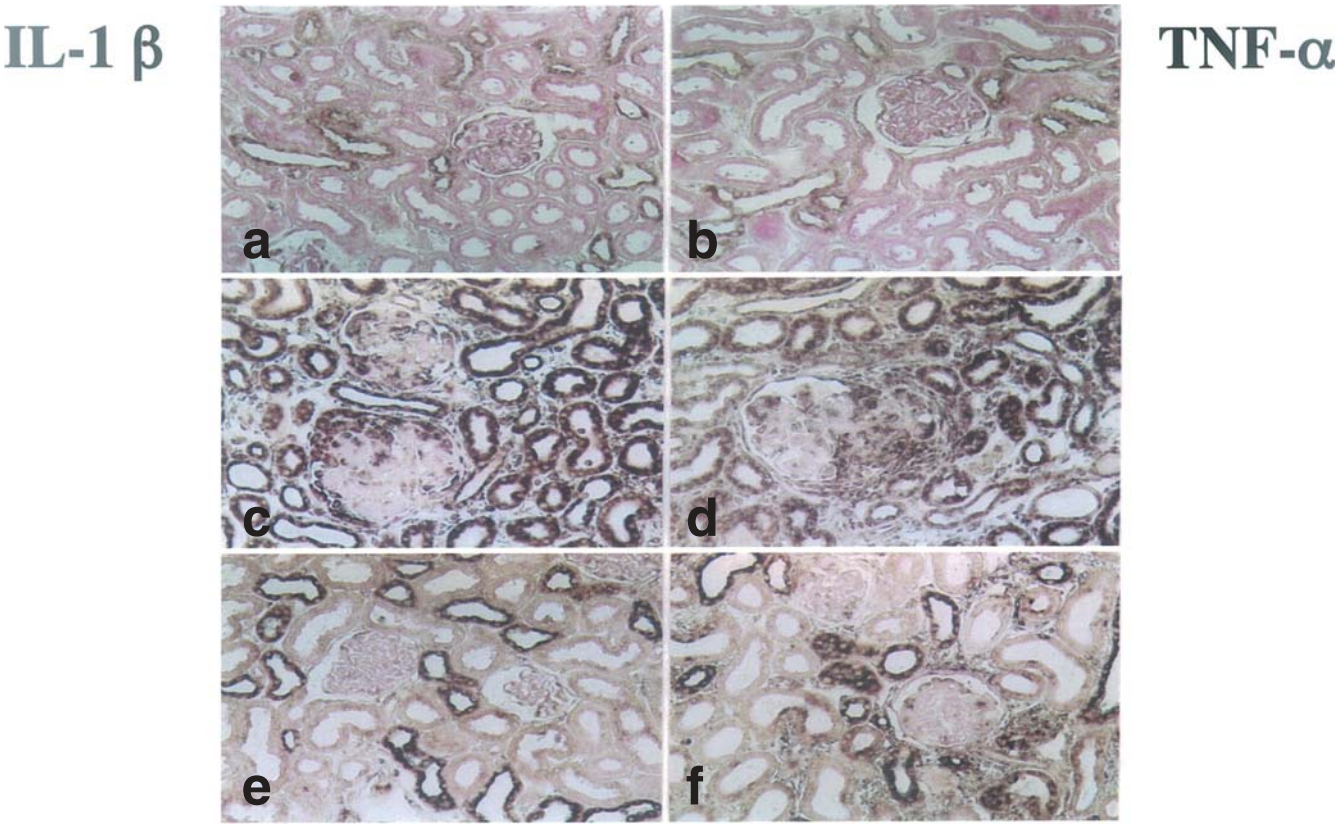

Fig. 8. IL-1β and tumor necrosis factor (TNF)-α mRNA expression in anti-GBM disease assessed by in situ hybridization. **a**,**b** Normal rat kidney. **c**,**d** SD decoy ODN-treated anti-GBM disease. **e**,**f** NF-κB decoy ODN-treated anti-GBM disease. Constitutive IL-1β mRNA expression was seen in normal rat kidney (**a**), which was substantially up-regulated in both glomeruli and tubules in SD decoy ODN-treated anti-GBM disease (**c**). Treatment of anti-GBM disease with NF-κB decoy ODN largely abrogated the increase in the glomerular IL-1β signal, and partially reduced the percentage of tubules expressing IL-1β (**e**). Constitutive TNF-α mRNA expression was also evident in normal rat kidney (**b**), and was markedly increased in SD decoy ODN-treated anti-GBM disease (**d**). NF-κB decoy ODN treatment substantially reduced TNF-α expression in anti-GBM disease (**f**)

mRNA expression. The up-regulation of TNF-α in anti-GBM disease was similar to that seen for IL-1β, and TNF-α expression was also significantly reduced by NF-κB decoy ODN treatment (Fig. 8). Leukocyte infiltration in the kidney was assessed by immunoperoxidase staining of tissue sections. The marked glomerular and interstitial leukocytic infiltration seen in the SD decoy-treated animals was significantly reduced by approximately 50% with NF-κB decoy ODN treatment (Fig. 9).

This study has demonstrated that the nuclear transcription factor NF-κB plays a key role in regulating the inflammatory processes underlying renal damage in experimental crescentic glomerulonephritis. Treatment with NF-κB decoy ODN caused a 50% reduction in proteinuria. Since NF-κB decoy ODN was transferred into one kidney only, this result documents a strong protective effect of decoy treatment on urinary protein excretion. This is consistent with the marked reduction in histological damage, cytokine expression, and leukocyte infiltration in the NF-κB decoy-treated kidney versus the untreated kidney. This study has also shown that NF-κB decoy ODN is a powerful inhibitor of the proinflammatory response in experimental crescentic glomerulonephritis. The results in this study may have implications for immune-mediated diseases generally. Given that blockade of IL-1β or TNF-α is an effective treatment for a variety of different models of immune-mediated disease, it is likely that NF-κB decoy ODN treatment will also be effective in such diseases.

Development of circular ribbon-type decoy ODN

The use of decoy ODN for reducing the *trans*-activity of transcription factors is an innovative strategy for gene therapy. However, the in vivo use of decoy ODN is hampered by nuclease digestion. Consequently, chemical modification procedures, such as phosphorothionation and methylphosphonation, were used to increase the stability of ODN against nuclease. A number of problems were encountered with these modified ODN, including sensitivity to RNase H, lack of sequence specificity, and immune activation.[84–88] To overcome these limitations, covalently modified ODN were developed by the enzymatic ligation of two identical molecules, thereby preventing degradation by exonuclease. These novel ODN possess increased nuclease resistance

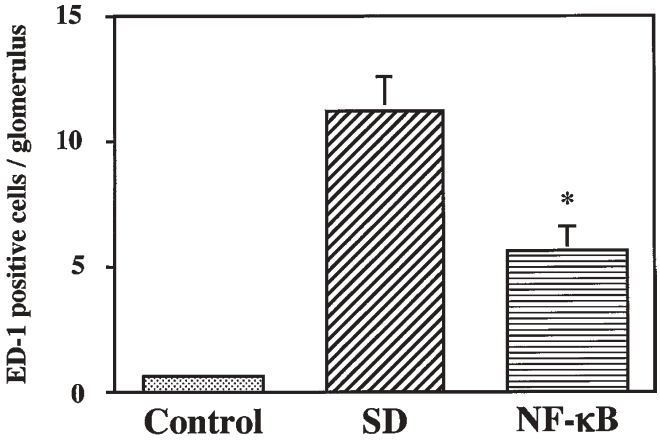

Fig. 9. Inhibition of renal leukocytic infiltration in anti-GBM disease by NF-κB decoy ODN. The number of ED-1 (+) leukocytes in glomeruli was quantitated on antibody-stained tissue sections. *Control*, normal rat kidney. *SD*, SD decoy ODN-treated anti-GBM disease. *NF-κB*, NF-κB decoy ODN-treated anti-GBM disease. Data are shown as mean ± SEM. $*P < 0.05$ vs. SD decoy ODN-treated anti-GBM disease, by ANOVA

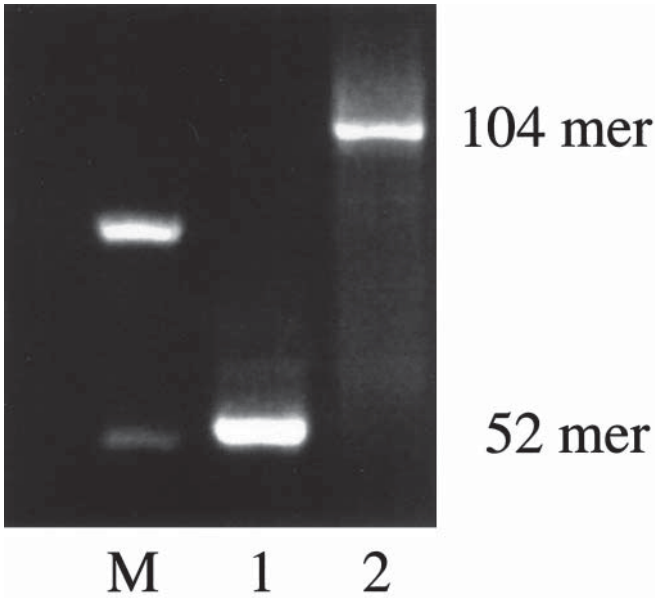

Fig. 10. Representative photograph of electrophoresis for ribbon-type decoy ODN. This photograph shows that almost 100% of ODN was annealed. *M*, size marker; *1*, identical ODN before annealing; *2*, identical ODN after annealing

and are transported more efficiently into cells than their chemically modified linear counterparts.[89–91] Using this concept, novel ribbon-type decoy ODN for E2F and NF-κB have recently been shown to be more effective than conventional decoy ODN.[92,93] We got several ideas from these reports, and are also trying to produce a similar ribbon-type decoy ODN. Ribbon-type decoy ODN is designed to have two circular loops and a stem containing the target transcription factor-binding site. Moreover, the sequence of ribbon-type decoy ODN is devised so that it may be composed of two pieces of identical sequence. ODNs were then ligated by T4 ligase. To increase the efficiency of ligation, we have tried annealing two pieces in many conditions, leading to the final condition that performs almost 100% ligation (Fig. 10). It is of interest that this newly developed ribbon-type decoy ODN was more easily transferred into MC in vitro than the conventional decoy ODN, which is a much smaller molecule with a lower molecular weight. On the other hand, it is true that we are encountering many issues which will need to be overcome for the further application of ribbon-type decoy ODN. The decoy ODN strategy is useful as a powerful tool in a new class of antigene strategies for gene therapy and also in the study of transcriptional regulation, in addition to the antisense strategy. The decoy strategy is particularly attractive for several reasons, as described previously.

Conclusion

The first federally approved human gene therapy protocol started on September 14, 1990, in ADA-deficient patients.[94,95] It is now more than 10 years since the start of the first trial, and more than 400 clinical studies of gene therapy have been undertaken. Their objectives are generally to evaluate (1) the in vivo efficacy of the gene transfer method, (2) the safety of the gene transfer method, and (3) the possible therapeutic efficacy. Although there are still many unresolved issues, human gene therapy is now a reality. Moreover, in 1996, the clinical application of decoy ODN against E2F was approved by the Food and Drug Administration (FDA) to treat neointimal hyperplasia in vein bypass grafts, which normally result in the failure of up to 50% of grafts within a period of 10 years.[96] Moreover, in Japan, a clinical trial of E2F decoy ODN for restenosis after percutaneous transluminal angioplasty (PTA) in patients suffering from ischemia in the legs has been started in Osaka University Hospital. This is the first trial in Japan of clinical gene therapy using decoy ODN for cardiovascular disease. Although there are still many outstanding issues in the clinical application of the ODN-based strategy, the utility of ribbon-type decoy ODN could be widespread as a tool for gene therapy in other diseases. Of course for an application to renal diseases, we also need to establish a novel safe and efficient transfer method. Taken together, now that gene therapy using ribbon-type decoy ODN appears to be close to reality, it is time to take a hard look at practical issues that will determine the real clinical potential.

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