Gene Therapy (2003) 10, 1910–1916

www.nature.com/gt

RESEARCH ARTICLE

Correction/mutation of acid α-D-glucosidase gene by modified single-stranded oligonucleotides: in vitro and in vivo studies

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Deficiency in acid α-D-glucosidase results in Pompe's disease. Modified single-stranded oligonucleotide (ODN) was designed to correct the acid α-D-glucosidase gene with a C1935→A (Asp→Glu) point mutation which causes a complete loss of enzymatic activity for glycogen digestion in the lysosome. The ODN vectors contained a stretch of normal oligonucleotide flanked by phosphorothioated sequences. The 25mer and 35mer ODNs were homologous to the target sequence, except for a mismatched base in the middle. The ODNs caused permanent and inheritable restoration of acid α-D-glucosidase activity in skin fibroblast cells carrying this mutation derived from a Pompe's disease patient. Gene correction was confirmed by amplification refractory mutation system-PCR (ARMS-PCR), restriction fragment length polymorphism (RFLP) and direct DNA cloning and sequencing. The increased acid α-D-glucosidase activity was detected using 4-MUG as the artificial substrate. The correction efficiency, ranging from 0.5 to 4%, was dependent on the length and polarity of the MSSOV used, the optimal design being a sense-strand 35mer ODNs. Repeated treatment of the mutant fibroblast cells with the ODNs substantially increased correction. We also constructed ODN vectors to trigger specific and in vivo nonsense mutation in the mouse acid α-D-glucosidase gene. The ODNs were in complex with YEEE-K_{18}, an asialoglycoprotein-receptor ligand tagged with polylysine and targeted to hepatocytes and renal cells in vivo through intravenous injection. The mutated genotype was detected in the liver and the kidney by ARMS-PCR and glycogen accumulation in the lysosome of the liver cells. The studies demonstrate the utility of single-stranded ODN to direct targeted gene correction or mutation in a human hereditary disease and in an animal model. Our data open the possibility of developing ODN vector as a therapeutic approach for treatment of human hereditary diseases caused by point mutation.

Gene Therapy (2003) **10,** 1910–1916. doi:10.1038/sj.gt.3302096

Keywords: *acid α-D-glucosidase; oligonucleotide; targeted gene correction; liver*

Introduction

Glycogen storage disease type II (GSD II), also known as Pompe's disease or acid maltase deficiency (AMD),[1,2] is genetically transmitted through autosomal recessive inheritance. [3] It is caused by a deficiency of acid α-D-glucosidase, a glycogen-degrading lysosomal enzyme. The enzyme catalyzes complete hydrolysis of glycogen by cleaving both α-1,4 and α-1,6 glycosidic linkages at acid pH liberating glucose to cytoplasm for reutilization. A defect in the enzyme results in lysosomal glycogen accumulation in almost all body tissues, with cardiac and skeletal muscle affected most seriously.[4,5] Based on clinical symptoms and the age of onset, such defects could be divided into three clinical types: infantile form, juvenile form and adult form. The infantile form is most severe and the patients die within a year.[6,7] There is currently no effective treatment for this fatal disease. Considerable efforts have been devoted to the development of gene therapy for Pompe's disease using viral vectors with only modest short-term successes due to limitations in transfection-targeting efficiency and immune responses.[8] Enzyme replacement therapy (ERT) for GSD II was reported, but ERT suffers from transient effectiveness and high cost.[9–11] Long-term correction of acid α-D-glucosidase deficiency requires a more permanent gene therapy protocol to provide stable expression of acid α-D-glucosidase, while circumventing problems associated with vector delivery or ERT.

Modified single-stranded oligonucleotide (ODN) vectors have been developed to target and trigger gene correction.[12–15] An ODN vector is a relatively short oligonucleotide containing 25–100 bases homologous to the target sequence except for a single mismatch; an ODN vector also contains a specific number of modified termini linkages. ODN vectors direct gene correction via DNA mismatch repair mechanisms.[12] Workers have shown that ODNs are effective in gene repair in cell-free extracts, cultured mammalian cells and yeast[13,14]

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Received 04 November 2002; accepted 08 May 2003

with comparable levels of gene repair to chimeraplasty,[15,16] an RNA–DNA double-stranded ODN. Advantages in the use of ODN vectors over the chimera approach include the ease of synthesis and purification of full-length and intact molecules.

A cytidine to adenosine (C1935→A) transversion causing a substitution of glutamic acid for aspartic acid at position 645 results in a complete loss of acid α-D-glucosidase activity.[17] This mutation is found in the major infantile form of GSDII prevalent in southern China and Taiwan.[18] Here, we investigated the use of modified single-stranded ODN vectors to correct this mutation in skin fibroblast cells derived from a patient. We also tested the use of YEEE-K_{18} as a carrier for *in vivo* delivery of ODN vector to mouse liver and kidney for gene correction. YEEE-K_{18} is a synthetic compound[19] containing a ligand of the asialoglycoprotein receptor[20] and a polysine chain for interacting with ODN. Our results demonstrate that ODN can be used to correct or create specific point mutations in the acid α-D-glucosidase gene both *in vitro* and *in vivo* and may be developed for use in gene therapy for Pompe's disease.

Experimental protocol

Oligonucleotide synthesis

Modified single-stranded ODN vector was synthesized in a 1.0 μmol scale on the ABI 391 DNA/RNA synthesizer by standard phosphoramidite procedure, except that the coupling time was increased to 3 min. Fluorescein-conjugated ODN was synthesized by incorporating fluorescein phosphoramidite. Full-length ODNs were purified by DMT-on method using OPC column and quantified by UV absorbance. The chemicals used for synthesis were purchased from Glen Research (Sterling, VA, USA).

Cell culture and transfection

Skin fibroblasts of a Pompe's disease patient were cultured in MEM medium containing 20% fetal calf serum, 1 mM nonessential amino acid, 2 mM L-glutamine, 100 U penicillin/streptomycin and 1 mM sodium pyruvate. For transfection experiments, cells were seeded at a density of 5×10^5 cells in a 6-cm plate 24 h prior to transfection. ODN (0.5 μM) was incubated for 10 min with 29–33 μg of lipofectin (Invitrogen, San Diego, CA, USA) in 470 μl of MEM. The lipofectin/DNA complex was added to the cells in wells containing 1 ml of MEM. After 3.5 h, cells were washed with phosphate-buffered saline (PBS) and fed with 5 ml medium. Cells were subcultured every 3 days. Total RNA and cell lysates were isolated 1 week later.

Detection of gene conversion by amplification refractory mutation system-PCR and restriction fragment length polymorphism analysis

Total RNA was isolated from the fibroblast using the RNA-Bee RNA isolation solvent (TEL-TEST, INC., TX, USA) and subjected to RT-PCR to generate a 742-bp fragment using the primers: Glu-5′-1, 5′AGACCGGCCAGCCGCTGA 3′ and Glu-3′-1, 5′ GCA GACTGAGCAGGCTGTTG 3′ with the following PCR program: 95°C 4 min; 55°C 30 s, 72°C 50 s, 95°C 30 s for 25 cycles; 55°C 1 min, 72°C 2 min. The PCR products were subjected to ARMS-PCR using the primers Glu-5′-2, 5′ GGACATGGTGGCTAGTTC 3′ and Glu-3′-pm, 5′CCAGGAAGCCGCAGGCG 3′ with the following program: 95°C 4 min; 62°C 30 s, 72°C 50 s, 95°C 30 s for 40 cycles; 62°C 1 min, 72°C 2 min. The underlined bases in Glu-3′-pm indicate mismatches with respect to the mutant gene sequence. The ARMS-PCR products (451-bp fragment) were analyzed by 1.5% agarose gel electrophoresis. For RFLP analysis, the PCR products were amplified by nested-PCR primers: Glu-5′-2 and Glu-3′-2, 5′ ^{32}P-GGCTGTTGTGGTTCCGCAT 3′ to generate a 529-bp fragment. The nested-PCR products were digested by *Aat*II and the reaction products were electrophoresed into a 7% polyacrylamide gel followed by autoradiographic detection.

Colony hybridization and sequencing

Nested-PCR product was ligated to the TA cloning vector pCR2.1 (Invitrogen, San Diego, CA, USA) and transferred into competent *Escherichia coli* for selection on an ampicillin LB plate. After incubation, the colonies were transferred onto 0.45 μm nitrocellulose membrane (MILLIPORE, MA, USA). Colony hybridization was carried out using the standard protocol.[21] The membranes were hybridized at 48°C for 24 h with a [^{32}P] end-labeled allele-specific oligonucleotide probe, Glu-p, 5′ CCCGGCTGCAGACGC 3′, in which the underlined 'G' was the corrected nucleotide. After hybridization and washing, the membranes were subjected to autoradiography. Positives clones were isolated and subjected to sequencing using the Glu-3′-2 primer in an automatic sequencer.

Acid α-D-glucosidase activity assay

Fibroblast cells were trypsinized from a Petri dish. The cells were washed twice with ice-cold PBS and resuspended in reaction buffer (40 mM Na_2HPO_4, 30 mM Na-citrate, pH 4.0). After sonication, the cell debris were removed by centrifugation (4°C, 13 000 rpm for 15 min). An aliquot of supernatant was subjected to determine protein content by BCA protein assay regent (Pierce, Rockford, IL, USA). The reaction buffer containing 100 μg of lysate protein and 25 μM 4-methylunbelliferyl-α-D-glucopyroside (4-MUG) (Fluka Chemical Corp, Ronkonkona, NY, USA) was incubated at 37°C for 2 h. The reaction was stopped by adding 1 ml of stop solution (0.4 M glycine-NaOH buffer, pH 10.6) and was measured by a fluorescence spectrophotometer (excitation at 365 nm and emission at 450 nm).

In vivo mutation of mouse acid α-D-glucosidase by ODN and mutation identification

ODN vector (10 nmol; see Figure 6a for sequences) was combined with YEEE-K_{18} (see Figure 6b) at a molar ratio of 1:2 in 200 μl of PBS. Aliquots were administered by tail vein injection into male BALB/*c* mice (25–30 g) at a dosage of 115 μg three times every second day. At 2 weeks after the last injection, the mice were killed and liver tissues were corrected for RNA isolation. RNA samples were subjected to RT-PCR using the primers mGlu-5′-1, 5′ AATGACCTGGACTACATGGA 3′ and mGlu-3′-1, 5′ TGAACCTGTACGGCTCCTGA 3′ with the following PCR program: 95°C 4 min; 55°C 30 s, 72°C 50 s, 95°C 30 sec for 25 cycles; 55°C 1 min, 72°C

2 min. The PCR products were then used as templates for ARMS-PCR to detect the introduced mutation as well as in nested-PCR as positive controls. The common 5′ primer of the nested-PCR and ARMS-PCR was mGlu-5′-2, 5′GACATGGTGTCTGAGTTCC 3′, the 3′ primers for the nested-PCR and ARMS-PCR were mGlu-3′-2, 5′ GGTCATTGTGGTTCCGCAT 3′ and mGlu-pm, 5′ AGCTCTTCTGACGTGTCACA 3′, respectively. The underlined bases in mGlu-pm indicate mismatches with respect to the normal gene sequence.

Liver and kidney tissues were fixed in formalin and then embedded in paraffin. The tissue sections were used for periodic acid–Schiff (PAS) staining which is specific for carbohydrate and gives a magenta color.

Result

Identification of a Pompe's disease patient with the C1935→A mutation

The acid α-D-glucosidase gene contains an *Aat*II restriction cleavage site at C1935,[18] and the C1935→A point mutation abolishes the site. We isolated total RNA from the skin fibroblasts of three Pompe's disease patients P6, P7 and P8, for amplification by nested RT-PCR to generate a 529-bp fragment (Figure 1a, upper panel). Upon *Aat*II digestion, the RT-PCR product of RNA from a normal individual resulted in 434-bp and 95-bp fragments. In contrast, the RT-PCR product from the patient remained intact indicating the presence of the C1935→A mutation (Figure 1a, lower panel). Patients P7 and P8 carry the mutation; however, P8 is heterozygous for the mutation while P7 carries the mutation in both chromosomes. The P6 patient manifested clinical symptoms of Pompe's disease and low-acid α-D-glucosidase activity in his fibroblast cells. The P6 patient may be carrying different mutations. Direct DNA sequencing of the RT-PCR product confirmed that P7 is a case of Pompe's disease homozygous for the C1935→A mutation (Figure 1b). The detection of the C1935→A mutation is consistent with the clinical manifestation and the almost complete loss of acid α-D-glucosidase activity[18] in this patient. In the skin fibroblast of P7, only 0.01% acid α-D-glucosidase activity of the normal individual was detected.

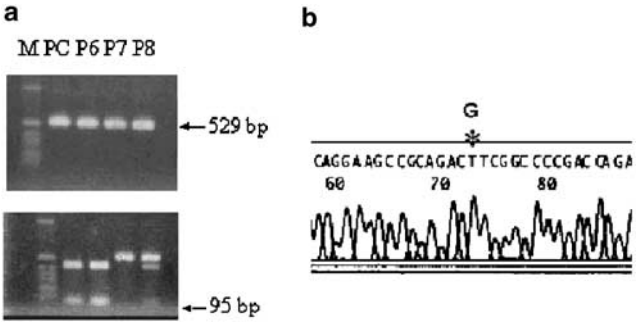

Figure 1 *Detection C1935→A mutation in Pompe's disease patients. (a) Nested RT-PCR product (529 bp) was amplified from total RNA of skin fibroblasts of Pompe disease patients by first and second primers for the human acid α-D-glucosidase gene (upper panel). The RT-PCR products were digested by AatII. Normal alleles generated the 434 and 95-bp bands. For the C1935→A mutant allele, the RT-PCR product remained unchanged due to the loss of the AatII site (lower panel). M, 1-kb DNA marker (GibcoBRL, Maryland); PC, a normal individual control, P6–P8, patient no. 6–8. (b) Direct sequencing of nested RT-PCR product of the acid α-D-glucosidase gene from patient P7 using the Glu-3′-2 primer. The asterisk indicates the site of substitution (C1935→A).*

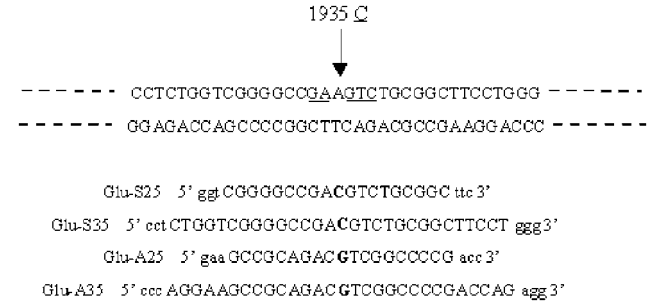

Figure 2 *ODN vectors of the acid α-D-glucosidase gene. A portion of the acid α-D-glucosidase gene making the C1935→A mutation site is shown at the top. AatII cutting site is underlined. The ODN sequences are shown below. Upper-case letters indicate normal DNA sequence and lower-case letters indicate phosphorothioate DNA sequence. The mismatched bases are in bold letters.*

Gene correction in cultured mutant skin fibroblasts

A modified single-stranded ODN vector was designed for correction of the C1935→A point mutation in the acid α-D-glucosidase gene (Figure 2). ODNs Glu-S25 and Glu-S35 are complementary to the transcribed strand; Glu-A25 and Glu-A35 are complementary to the non-transcribed strand of the DNA. Skin fibroblast cells of patient P7 were transfected with ODN vectors using lipofectin as a carrier. At 7 days after transfection, total RNAs from transfected and untransfected fibroblasts were amplified by RT-PCR. To detect the correction, the products were further analyzed by the ARMS-PCR. The 3′ primer (Glu-3′-pm) designed for the ARMS-PCR carried only one mismatched base relative to the normal sequence and two mismatches relative to the C1935→A mutated sequence. The primer was designed to differentiate between the normal from the C1935→A mutated sequence. If the C1935→A mutation had been corrected by the introduced ODNs, the primer would generate a 451-bp DNA fragment in ARMS-PCR analysis. As shown in Figure 3a, bands of different intensities corresponding to the size of the normal band were detected when different ODN vectors were used, indicating that different extents of correction had indeed occurred. The data further show that the 35mer ODNs were more effective than the 25mer ODNs (Figure 3a, lanes 4 and 6 *versus*. lanes 3 and 5) and that the sense-strand ODNs seemed better than the antisense strand ODNs (Figure 3a, lanes 4 and 6). The differences were not due to intracellular concentration of ODN. Using lipofectin as a carrier, no detectable difference was found for the internalization of fluorescein-labeled ODNs when inspected under a fluorescence microscope.

We confirmed the ARMS-PCR results by RFLP analysis. To increase sensitivity, the RT-PCR product was further amplified by nested-PCR using the Glu-5′-2 primer and the end-labeled Glu-3′-2 primer and *Aat*II restriction digestion. The detection and quantification of the expected 95-bp band confirmed the ARMS-PCR results, that is, the 35mer was a better corrective construct than the 25mer and that the sense strand (Figure 3b, lane 3) works better than the antisense design in our case (Figure 3b, lane 1).

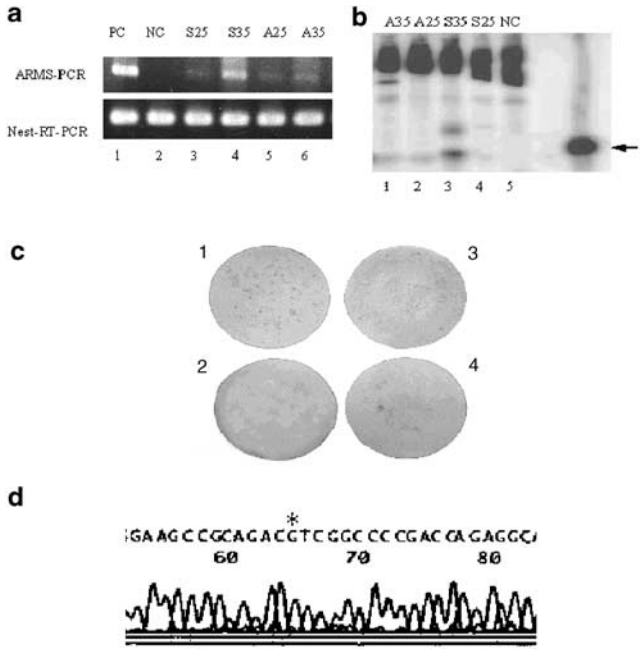

Figure 3 *Detection of gene correction in cultured C1935→A mutant skin fibroblasts. (a) ARMS-PCR analysis of mutant skin fibroblasts treated with different ODNs. (b) RFLP analysis of cDNA derived from total RNA isolated from the mutant fibroblast cells treated with ODNs. The arrow designates the 95-bp size marker. PC: fibroblast of normal individual, NC: P7 fibroblasts without transfection; S25, S35, A25 and A35 represent P7 fibroblasts treated with Glu-S25, Glu-S35, Glu-A25 and Glu-A35, respectively. (c) Autoradiograms for colony hybridization are shown, the nested-PCR products were cloned. Colony hybridizations were conducted as described in 'Experimental protocol'. 1: DNA from normal individual, 2: DNA from P7 fibroblasts 3 and 4: DNA from P7 fibroblasts treated with Glu-S35 and Glu-A35, respectively. (d) DNA sequence analysis of the corrected acid α-D-glucosidase gene in a positive clone isolated colony hybridization. The asterisk designates the corrected base.*

For further analysis, the nested-PCR products were cloned. Autoradiograms for colony hybridization are shown in Figure 3c. Five percent of the colonies elicited positive signals for the S35-treated group and 1.5% for the A35-treated group. Inserts of 10 positive clones were sequenced, 2/3 of which showed the corrected sequence (Figure 3d). No other nucleotide alterations were observed in the sequenced region (data not shown).

The acid α-D-glucosidase activity of the transfected fibroblasts was assayed using 4-MUG as the artificial substrate. The enzyme activity in the treated cells was elevated from the original 0.01% to ~0.5–4% after transfection treatment with different ODNs (Figure 4).

To enhance the correction efficiency, we repeatedly treated the C1935→A fibroblasts with ODNs three times. Total RNAs were isolated and subjected to similar RT-PCR and *Aat*II analysis. After repeated ODN treatment, densitometric quantification of the relative band intensities indicates that repeated ODN treatment substantially increased correction in both the sense and antisense ODN cases (Figure 5).

In vivo mutagenesis of the acid α-D-glucosidase gene in mouse liver and kidney

To test the use of ODN vectors in inducing precise nucleotide change *in vivo*, we constructed ODNs to create a nonsense mutation in the mouse acid α-D-glucosidase gene (Figure 6a). The ODNs were delivered using the carrier–YEEE-K_{18} (Figure 6b), a ligand of asiologlycoprotein receptor tagged with polylysine. By tail vein injection, fluorescein-labeled ODN was administered to mice. The mice were killed 1.5-h postinjection and perfused with PBS. The fluorescein-labeled ODN was found to accumulate in the liver and the kidney (Figure 7a). Little or no fluorescence was detected in the other major organ analyzed (data not shown). For detection of the mutation, ODN/YEEE-K_{18} was administered *in vivo* by tail vein 3 times every second day. At 2 weeks after the final injection, RNAs were isolated from the liver and the kidney for RT-PCR and mutation was assayed by ARMS-PCR (Figure 8). The results show that RNAs of the liver and the kidney of the treated mice generated a 451-bp fragment by ARMS-PCR, indicating that the ODNs had caused a site-specific mutation in these organs.

The samples of livers and kidneys were paraffin-embedded and sectioned for PAS staining. In acid α-D-glucosidase-deficient cells, glycogen is expected to accumulate in lysosomes in 2 weeks. Such glycogen accumulation was found only in the liver of transfected mice, especially around the blood vessels (Figure 7b).

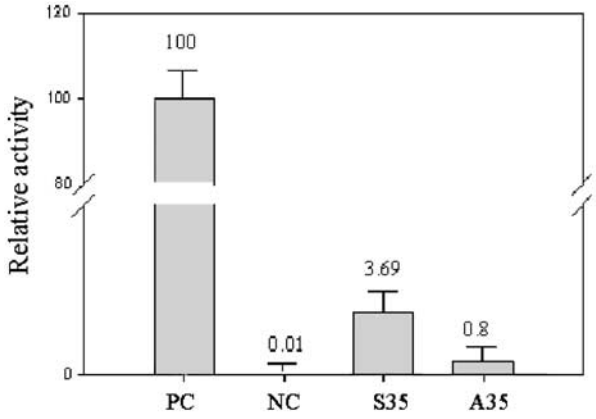

Figure 4 *Acid α-D-glucosidase enzyme activity of C1935→A mutant fibroblasts after ODN treatment. The activity of different ODN-treated fibroblasts and the normal individual (PC). The experiments were triplicated. Numericals indicate the mean values obtained.*

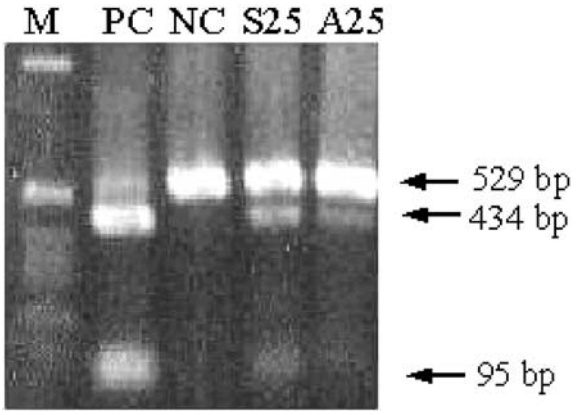

Figure 5 *Restriction analysis of cDNAs generated from total RNA isolated from the skin fibroblasts of a Pompe's disease C1935→A patient after repeated treatment with ODN, see legend of Figure 3 for lane labels.*

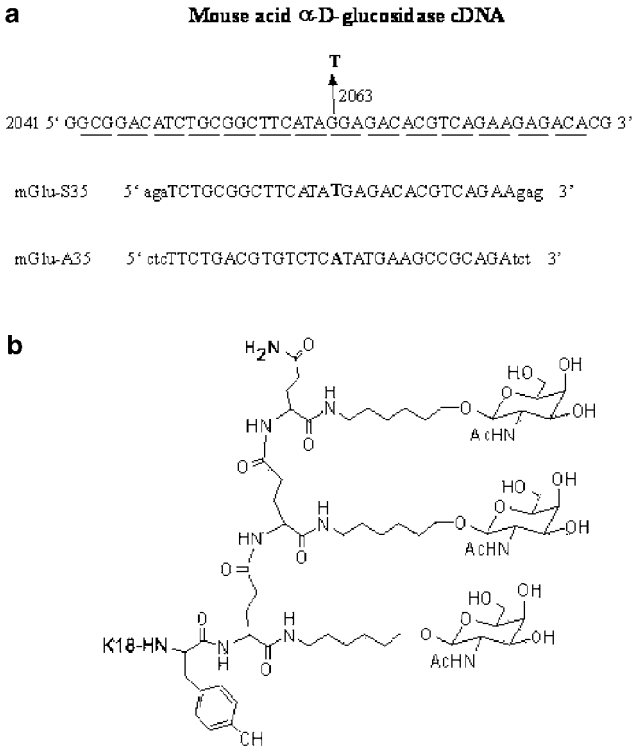

Figure 6 *In vivo introduction of a nonsense mutation in the mouse acid α-D-glucosidase gene. The translational reading frame is underlined. The stop codon (TGA) created by the G2063→T mutation is shown. The ODN vector, mGlu-S35 and mGlu-A35 used for introducing this mutation are shown below. Bold letters indicate the mismatched bases. Lower-case letters indicate phosphorothioate nucleotides. (b) Structure of YEEE-K_{18}. 'K_{18}' is the 18mer lysine chain. The synthesis of YEEE was described previously.*[19]

Glycogen accumulation in the kidney was, however, less obvious (data not shown).

Discussion

In this study, PCR-based detection was chosen because the cells we used were hard-to-obtain primary fibroblasts constrained by limited passage capacity. To circumvent the possible event of priming by degraded ODN during PCR, the RNAs were extracted from the cells 1 week after transfection in order to clear the ODN and surviving mRNA in the cells. To prove that no such ODN had contributed to the PCR, we did the following experiment: For RNAs derived from the treatment of sense ODN, 3′ primer was added after the RT reaction; for RNAs derived from the antisense ODN treatment, 5′ primer was added. PCR was carried out under the same conditions. No PCR product of the expected size was

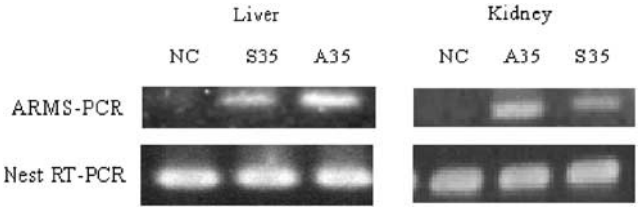

Figure 8 *Detection of in vivo mutation of the acid α-D-glucosidase gene in the liver and kidney of treated mice. Mice were treated with mGlu-S35/YEEE-K_{18} (S35), mGlu-A35/YEEE-K_{18} (A35), or YEEE-K_{18} only (NC) by i.v. three times every second day. At 2 weeks after the last treatment, mice were killed. Total RNAs were subjected to ARMS-PCR. The nested RT-PCR products were used as internal controls.*

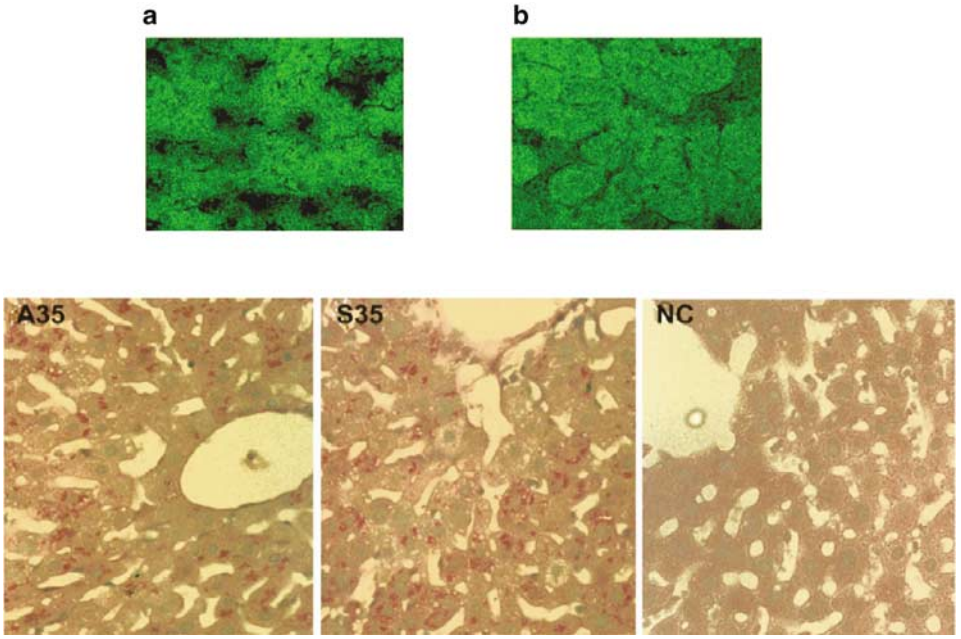

Figure 7 *Microscopic photos of the tissue biopsies. (a) In vivo delivery of fluorescein-labeled ODN/ YEEE-K_{18} complex to mice by tail vein injection. Mice were killed 1.5 h postinjection. Frozen sections of the liver (a) and kidney (b) biopsies were inspected by fluorescence microscope. (b) Mice were treated with mGlu-S35/YEEE-K_{18} (S35), mGlu-A35/ YEEE-K_{18} (A35), or YEEE-K_{18} only (NC) three times every second day. At 2 weeks after the last treatment, the mice were killed. The liver biopsies were fixed by formalin and embedded in paraffin. The liver biopsy sections were stained by PAS to detect glycogen accumulation. Pictures shown are views of cell tissues around blood vessels.*

found. Under the same consideration, the RNAs were extracted from the livers 2 weeks after the last injection in order to clear the ODN and surviving mRNA in the cells (as well as to allow mutated cells to accumulate glycogen).

In our study, we demonstrated that single-stranded ODN vector accurately corrected the targeted C1935→A point mutation of the acid α-D-glucosidase gene in fibroblast cells and resulted in increased enzyme activities from 0.01% to about 4%. Repeated treatment of the fibroblasts with ODN further substantially increased the correction percentages. Recently, Goukassian *et al* found that small single-stranded DNA fragments activate p53, enhance DNA repair and compensate for age-associated decline in DNA repair capacity in primary cultured fibroblast.[22] It may be the cause for the enhancement. The targeted correction is highly specific since no other nucleotide changes were found in the 0.5-kb fragment harboring the site of correction. For *in vivo* tests, an ODN/YEEE-K_{18} complex was used to create a nonsense mutation in the acid α-D-glucosidase gene in mouse liver and kidney. Asialoglycoprotein receptor of the liver is known to bind and endocytose old serum asialoglycoprotein[23] and has been utilized for liver-targeting delivery.[24] In the kidney, the occurrence of such an event is more controversial. However, Seow *et al*[25] have recently demonstrated the existence of asialoglycoprotein receptor in human renal tubular epithelial cells. Results presented in the study are consistent with the existence of such a receptor in the kidney. Ablation of acid α-D-glucosidase activity by MSSOV had resulted in glycogen accumulation in the lysosome of the mutated liver cells. However, glycogen accumulation was not observed in the kidney probably due to differences in the nature of kidney cells. Our results implicate that the modified single-strand ODN/YEEE-K_{18} system is applicable to correct (or mutate) gene in both the liver and kidney.

Yoon and colleagues have previously addressed strand bias in gene repair using MSSOV and found that antisense (ie ODN complements to the nontranscribed strand of DNA) is 1000-fold more efficient than sense constructs.[14] Liu *et al*[15] also showed that antisense exhibited a five- to six-fold increase in correction efficiency. In contrast, Yamamoto's study[13] in transformation of yeast by oligonucleotides showed that the sense strand affected the target sequence 50–100-fold more effectively than antisense oligonucleotides. In our system, the sense strand worked several fold better than the antisense strand. The discrepancy may have resulted from differences in experimental conditions in target genes, lengths and modifications of the ODNs. We cannot rule out the sequence factor *per se* since the sense and the antisense sequences are always different.

Gene correction of liver cells is clinically advantageous. Liver is the biggest and most important organ in mammals responsible for metabolism and secretion of many enzymes. Many liver diseases are caused by point mutations, including hemophilia A and B, familiar hypercholesterolemia, α-1-antitrypsin deficiency and the Crigler–Najjar syndrome. Moreover, liver has the ability to regenerate and is suitable for *ex vivo* cultivation. This characteristic makes liver a favorable target site for gene delivery and thus correction. Chen and colleagues[26,27] have developed a new strategy called hepatic gene therapy for treatment of GSD II using adenovial vectors for transduction of hepatocytes. As the liver is a secretory organ, it could produce and secrete large amounts of the acid α-D-glucosidase protein into the bloodstream for reabsorption by lysosomes of multiple muscle groups to incur cross-correction in remote target organs. However, expression of the acid α-D-glucosidase gene in adenoviral vector-transduced hepatocytes is transient and would be expected to induce immune responses. Gene correction directed by ODN/YEEE-K_{18} system, however, is permanent and the expression is under the control of the original promoter of the target gene. Since only small molecules are introduced, the problems of immunoresponses are largely circumvented. A permanent reservoir of acid α-D-glucosidase could be created in treated individuals.

MSSOVs are easily synthesized and purified. Oligonucleotides are relatively less toxic to human. The results of our experiments are promising for clinical application of ODN and ODN/YEEE-K_{18} for gene therapy of disorders caused by point mutations.

Acknowledgements

This work was supported by NSC grants. We thank Dr Kong-Bung Choo for critically reading the manuscript.

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