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Selective Inhibition of α-Galactosidase A with Antisense Oligodeoxynucleotide in Mesangial Cells: A Renal Cellular Model for Fabry Disease

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Abstract

Background and Aims: Fabry disease is an X-linked lysosomal storage disease resulting from deficient activity of the enzyme α-galacotsidase (α-Gal) A. Accumulation of glycosphingolipids, especially globotriaosylceramide, leads to various organ damage in Fabry disease. Recently, replacement with recombinant α-Gal A has become available for the treatment of this disease. However, the pathogenic mechanism of this disease, which is the accumulation of glycosphingolipids, is still unknown. Understanding the pathogenesis of Fabry disease may allow more efficient treatments. We examined whether the selective inhibition of α-Gal A with phosphorothioate antisense oligonucleotides could be used as a renal cellular model for Fabry disease.

Methods: Phosphorothioate antisense oligonucleotides designed to hyrbridize to sites on the human α-Gal A mRNA were tested for inhibition of α-Gal A expression in human mesangial cells. α-Gal A activity was measured using an artificial substrate, 4-methylumbelliferyl-α-D-galactoside.

Results: Two antisense oligonucleotides selectively inhibited α-Gal A activity to below 20% of the mean control activity. These oligonucleotides did not affect other lysosomal enzyme activities.

Conclusions: These data indicate that phosphorothioate oligonucleotides are capable of selectively inhibiting α-Gal A expression. It may be a useful model for renal mesangial cells in Fabry disease.

Keywords: Fabry Disease, α-galactosidase A, Antisense Oligodeoxynucleotide, Globotriaosylceramide,

Introduction

Fabry disease, an X chromosome-linked disorder caused by a genetic deficiency of lysosomal α-galactosidase (α-Gal) A, leads to the systemic accumulation of metabolic intermediates, i.e. glycosphingolipids, particularly globotriaosylceramide (1). In classically affected hemizygous male patients,

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the systemic accumulation of globotriaosylceramide causes a variety of clinical manifestations such as pain and paresthesias in the distal extremities, angiokeratoma, hypohidrosis, corneal opacity, cardiac dysfunction, and systemic vasculopathy (1). Renal involvement is one of the most serious complications of this disease, and often determines prognosis. The prevalence of Fabry disease may be more common among dialysis patients than previously believed (2, 3). Glycosphingolipids accumulate mainly in the epithelial cells of the glomerulus, the distal tubules, and the proximal tubules, as well as interstitial histiocytes and interstitial cells. This leads to proteinuria and loss of renal concentrating ability, causing polyuria and polydipsia. The mesangial cells and their surrounding-matrix material constitute the mesangium, which is separated from the capillary lumen by endothelium. It participates in the control of glomerular filtration. However, the influence of the accumulation of glycosphingolipids on mesangial cells has not yet been elucidated.

Recently, the safety and effectiveness of recombinant α-Gal A as replacement therapy for Fabry disease have been reported (4, 5). To confirm its efficacy, understanding its pathogenesis would be helpful.

Antisense oligonucleotides are short synthetic oligonucleotides designed to hybridize to RNA that encodes a protein of interest. These have been utilized to inhibit the expression of a number of cellular and viral proteins (6, 7). We designed antisense oligonucleotides to α-Gal A in order to make a model of Fabry disease so that its renal mesangial cellular pathogenesis could be understood better.

Materials and Methods

Cells and reagents

Human mesangial cells (Clontec, San Diego, CA) were cultured in RPMI 1640 made containing 20%100 U/ml penicillin and 100μg/ml streptomycin. The cells, between the second and seventh passage, were plated in 35-mm dishes. Cationic lipid molecule/DOPE solution (TfxTM-50) was purchased from Promega (Madison, MI).

Oligonucleotide synthesis

Phosphorothioate oligodeoxyribonucleotides were synthesized on an automated DNA synthesizer (Applied Biosystems Model 380B). The oligonucleotides were analyzed by polyacrylamide gel electrophoresis on denaturing gels and judged to be at least 85% full-length material. Oligonucleotides were purified by high performance liquid chromatography (HPLC) as previously described (8).

Oligonucleotide treatment

Cells were washed in a serum-free medium prewarmed to 37 ºC. Cell growth media (RPMI 1640 containing 20% fetal bovine serum) were added to each well of the plate (400 μl). Oligonucleotides were sterilized by centrifugation through 0.2 μm Centrex cellulose acetate filters. Oligonucleotides were added to wells with a cationic lipid molecule/DOPE solution (TfxTM-50 Promega, Madison, WI) to yield a final concentration of oligonucleotide as 10 μM. The media were changed for every 12 hr at 37 ºC for 96 hr. Sense and scramble oligonucleotides were used as controls.

Enzyme assay

After 96 hr, the cells were trypsinized and used to measure α-Gal A and β-hexosaminidase (β-Hex, control enzyme) activity using an artificial substrate, 4-methylumbelliferyl- α-D-galactoside, as described by Mayes et al. (9).

Results

Eleven synthetic oligonucleotides were designed n ear the AUG translation initiation codon of the α-Gal A mRNA (Table 1). Number 1 and 8 selectively inhibited α-Gal A expression of human mesangial cells (Table 1).

Table 1. αGal A antisense oligonucleotide

Oligonucleotide	Length	Sequence (5'-3')
1	24	**CAT**TGTCACGGTGACCGGACAGCA
2	24	G**CAT**TGTCACGGTGACCGGACAGC
3	24	TG**CAT**TGTCACGGTGACCGGACAG
4	24	CTG**CAT**TGTCACGGTGACCGGACA
5	24	GCTG**CAT**TGTCACGGTGACCGGAC
6	24	CAGCTG**CAT**TGTCACGGTGACCGG
7	24	CCTCAGCTG**CAT**TGTCACGGTGAC
8	24	TCCTCAGCTG**CAT**TGTCACGGTGA
9	24	GTTCCTCAGCTG**CAT**TGTCACGGT
10	24	TGGGTTCCTCAGCTG**CAT**TGTCAC
11	24	TTCTGGGTTCCTCAGCTG**CAT**TGT

Table 2. α-Gal A activity

Oligonucleotide	α-Gal A activity	β−Hex activity
1	14.1 (20)	2407
2	46.3 (66)	3013
3	63.8 (91)	6160
4	60.9 (87)	6172
5	66.6 (95)	3010
6	54.7 (78)	4107
7	79.9 (114)	4244
8	12.6 (18)	2109
9	45.6 (65)	2681
10	65.9 (94)	3156
11	70.1 (100)	2792

α-galactosidase (α-Gal) A activity, nmol/h/mg; **protein β-Hexosaminidaseactivity,**nmol/h/mg; **protein parenthesis,** percetage of controls.

These oligonucleotides reduced the activity of α-Gal A to less than 20 % that in the control cells. These did not affect β-Hexosaminidase activity, a control enzyme (Table 2). Sense and scramble oligonucleotides failed to inhibit α-Gal A expression (data not shown).

Discussion

The mesangial cells and their surrounding matrix material constitute the mesangium, which is separated from the capillary lumen by endothelium. It provides structural support for the glomerular capillaries. The contractile property of mesangial cells participates in the control of glomerular filtration. The phagocytic properties of the mesangial cells are well documented. The phagocytosed material may be cleared from the mesangium by cell-to-cell transport of the glomerular tuft. We demonstrated that antisense oligonucleotides can inhibit α-Gal A mRNA expression in mesangial cells. It may be useful to further understand the pathogenesis of renal failure in Fabry disease. Oligonucleotides may inhibit the expression of a target protein by several mechanisms. These include the prevention of new

protein synthesis by translational arrest, the inhibition of mRNA maturation by masking sequences required for the formation of the spliceosome, the inhibition of mRNA transport out of the nucleus, the inhibition of gene transcription by forming a triple helix structure, or other unidentified mechanisms (10, 11). Two antisense oligonucleotides hybridized to the AUG translation codon. Targeting the AUG codon would be expected to mask the ribosome recognition site and prevent the formation of the translation complex.

Antisense oligonucleotides are capable of specifically reducing the expression of α-Gal A to below 20% of the control in a mesangial cell culture system. The variation in the β-Hex was within normal range (12). This model is easy to study in comparison with using knock out animal models. Further optimization of the antisense oligodeoxynucleotides (sequence, chemistry and uptake) and treatments (dose, schedule) may provide increased inhibition. Nonetheless, the inhibition of α-Gal A expression with antisense oligonucleotides represents a novel approach for understanding the pathogenesis of Fabry disease.

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Conflict of Interest

None declared.

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