A new approach for innovative nanomedicine of type 2 diabetes in combination with DNA microarray and in vivo siRNA delivery

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ABSTRACT SUMMARY
Searching for a new therapeutic target is very important for the drug development. We previously found that monoacylglycerol O-acyltransferase 1 (Mogat1) was highly expressed in diabetic mice liver by DNA microarray analyses. However, in vivo functional analyses were restricted because there were little efficient in vivo siRNA delivery systems. In this study, an in vivo siRNA delivery system has been developed in our laboratory to validate the efficacy of hepatic Mogat1 for a target gene of type 2 diabetes. We revealed that hepatic Mogat1 silencing essentially inhibited the progression of the type 2 diabetes. This study could contribute to the next generation of the innovative drug development.

INTRODUCTION
In vivo functional analysis of a therapeutic candidate gene is very important for an innovative nanomedicine. By using promising new biotechnologies such as microarray, proteomics, next generation sequencing, and other platforms, we can obtain many therapeutic candidate genes. However, all of them are not really related to diseases. Though knockout mice and low molecule inhibitor are now used to observe an in vivo phenotypic change when a candidate gene is inhibited, these ways are time-consuming and expensive.

To solve this issue, we herein propose an innovative methodology for developing medicine. In our laboratory, by using DNA microarray analyses, we showed that the expression level of a monoacylglycerol O-acyltransferase1 (Mogat1), an enzyme involved in triglyceride (TG) synthesis and storage, was elevated in livers of diabetic mice compared to normal mice. In addition, a non-viral in vivo siRNA delivery system (MEND) that can effectively silence a specific gene in liver has been developed. In this study, we demonstrate the usefulness of the method in which omics technology and siRNA delivery are assembled.

EXPERIMENTAL METHODS
Quantitative RT-PCR (qRT-PCR) analyses were performed to measure hepatic Mogat1 mRNA levels in KKAy and db/db mice (type 2 diabetes model) and diet-induced obesity (DIO) mice (obese model). We also checked tissue expressions of Mogat1 in pre-diabetic KKAy mice.

siRNA was formulated into lipid nanoparticles composed of a pH sensitive cationic lipid, YSK05. The lipid nanoparticles contained YSK05, cholesterol and PEG-dimyristoylglycerol in molar ratio 70:30:3. Anti luciferase (luc) siRNA was used as a negative control. The size of MEND was about 80 nm with neutral surface charge with dynamic light scattering.

The lipid nanoparticles encapsulating Mogat1 or luc siRNA was injected into five-weeks-old KKAy mice via the tail vain 4 times every 5 days. Each injection dose was 2 mg/kg. Hepatic Mogat1 knockdown was measured by qRT-PCR analyses. Relative Mogat1 expression level was normalized to ActB expression level. The value of control mice was set as 1. The blood glucose level was monitored every day after 4th injection, the liver and serum were collected 21 days after the first injection. Serum insulin, adiponectin, and some lipids levels were measured, and the volume of intra hepatic lipid was validated by confocal imaging study. To ensure that our siRNA delivery system was safety, hepatic and kidney toxicities were evaluated.

RESULTS AND DISCUSSION
Tissue expression analysis of Mogat1 and Mogat2 showed that in pre-diabetes KKAy, high expression of Mogat1 and Mogat2 was observed in kidney and small intestine, respectively, but Mogat1 and Mogat2 expression in liver were very low. However, the hepatic Mogat1 expression was increased at post-diabetes stage; 25-fold higher in 11w KKAy mice, 31-fold higher in 10w db/db mice than control mice respectively. Moreover, obese DIO mice had 2.7-fold higher expression level than C57BL/6J mice (Figure 1). These results suggested that hepatic Mogat1 gene expression level could be something associated with the progression of type 2 diabetes. Accordingly, we next examined a therapeutic effect of hepatic Mogat1 gene silencing in pre-diabetic KKAy mice.

Next, the phenotypic analysis was performed by using the siRNA delivery system. First, the silencing effect after injection of siRNA loaded YSK-MEND was evaluated. Mogat1 siRNA loaded YSK-MEND treatment resulted in 72% reduction of hepatic Mogat1 expression compared with luc siRNA treatment 6 days after the last injection (Figure 2). The expected biological effects resulting from Mogat1 mRNA silencing include normal blood glucose level and other serum hormone such as insulin and adiponectin. Then the hepatic Mogat1 silencing caused significant reduction of normal blood glucose level for the 5 days in a row after the last injection (Figure 3). In addition, serum insulin level was
mildly reduced, indicating that normal blood glucose level was better controlled by low level of insulin. Moreover, serum adiponectin, an indicator of overall insulin sensitivity, was significantly increased. These results suggested that the durable suppression of hepatic Mogat1 mRNA caused essential improvements of insulin resistance, leading to the drastic preventing effects for type 2 diabetes.

![Figure 1](image1.png)

**Figure 1** The hepatic Mogat1 expression in KKAy, db/db and DIO mice. Relative Mogat1 expression level was normalized to Actb expression level. The value of control mice was set as 1. These data represented means ± S.D. **p<0.01, ***p<0.005 vs. control mice (Student’s t-test).**

![Figure 2](image2.png)

**Figure 2** Hepatic Mogat1 silencing effect 6 days after the 4th injections. Relative Mogat1 expression level was normalized to Actb expression level. **p<0.01 vs. luc (Student’s t-test).**

Next, we evaluated amounts of the ectopic fat deposition in liver, because of Mogat enzymes affect TG production, which is the final product of the monoacylglycerol pathway. A confocal imaging study showed that hepatic Mogat1 silencing had preventive effect on the increasing of the hepatic ectopic fat accumulation. In particular, hepatic TG and non-esterified fatty acid contents were both decreased. Moreover, the serum lipid parameters were also measured, because hyperlipidemia is likely to be associated with type 2 diabetes. The serum values of TG and cholesterol were significantly reduced in groups treated with Mogat1 siRNA. Furthermore, an increase in body weight was suppressed in groups treated with Mogat1 siRNA. These results indicated that the long term Mogat1 gene silencing has therapeutic effect not only for the prevention of type 2 diabetes but also fatty liver and obesity as well.

![Figure 3](image3.png)

**Figure 3** Normal blood glucose level was monitored after the 4th injections. These data are represented means ± S.D. *p<0.05, **p<0.01 vs. luc (Student’s t-test).

Finally, the hepatic and kidney toxicities caused by repeated injections of siRNA encapsulated in YSK-MEND were evaluated, since liver and kidney were considered to be major clearance organs for liposomal carriers. Prominent liver damage was undetected 1 day after the injection. In addition, serum creatinine and lactate dehydrogenase (LDH) level, an indicator of kidney and overall organ toxicity respectively, was both acceptable values. Furthermore, the monitoring of serum AST and ALT (the indicator of hepatic toxicity) levels showed that cumulative and acute toxicity was not observed after each injection.

**CONCLUSION**

The hepatic Mogat1 knockdown caused essential improvements in normal glucose level, accompanied by significant reduction of hepatic and serum lipid parameters and body weight. These results clearly demonstrate that hepatic Mogat1 gene is attractive therapeutic target for type 2 diabetes as well as fatty liver, hyperlipidemia, and obesity. The in vivo siRNA delivery system (YSK-MEND) used in this study had no acute liver and kidney toxicities after the single and repeated injections. In conclusion, the YSK-MEND system loaded with Mogat1 siRNA promises to be an innovative nanomedicine for treating metabolic diseases.

**REFERENCES**


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