Title

The systemic administration of an anti-miRNA oligonucleotide encapsulated pH-sensitive liposome results in reduced level of hepatic microRNA-122 in mice

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Figure S1 Characterization of the AMO122-YSK05-MEND. (a) Gel electrophoretic analysis of the encapsulation of AMO122 in YSK05-MEND. Free AMO122 and AMO122-YSK05-MEND were treated with Triton X-100 to disrupt the lipid envelope structure. Samples were subjected to agarose gel electrophoresis. A 20 bp DNA ladder (Takara) was applied in lane 1. (b) Determination of pKa by TNS. YSK05-MENDs were diluted to 30 μM total lipid in buffers containing 6 μM TNS at various pH levels. The highest fluorescence was assigned as 1.0 and the relative fluorescent intensities are represented. Data represent the mean value ± SD (n=3). Curve fitting (gray line) was calculated using Sigmaplot 12.
The effect of AMO10b encapsulated in the YSK05-MEND (AMO10b-YSK05-MEND) on the expression of miR-122 and target genes.

AMO10b (5’-CsCsAsAsAsUsUsCsGsGsUsUsCsUsAsCsAsGsGsGsUsA-3’) was purchased from Hokkaido System Science (Sapporo, Japan) (2’-OMe-modified nucleotides; subscript ‘s’ represents a phosphorothioate linkage). The diameter, pid, zeta-potential, and recovery and encapsulation efficiency of AMO10b formulated YSK-5-MEND (AMO10b-YSK05-MEND) were 67 ± 5 nm, 0.181 ± 0.04, 0,1 ± 2.4 mV, 92 ± 2% and 95 ± 7%, respectively. The characteristics of AMO10b-YSK05-MEND were comparable to that encapsulating AMO122. Either AMO122-YSK05-MEND or AMO10b-YSK05-MEND was successively administered via the tail vein of ICR mice at a dose of 1 mg/kg at day 0, 2 and 4. At day 6, liver was collected and subjected to real-time PCR for the determination of the expression level of miR-122 and target genes as described in the materials and methods section. The administration of AMO10b-YSK05-MEND showed no change in the expression of miR-122 and target genes, while AMO122-YSK05-MEND reduced the expression of miR-122 and enhanced the target AldaA, Bckdk and Ndrg3. These results indicate that miR-122 inhibition and the subsequent enhancement of target genes occurred in an AMO122 sequence.
specific manner. Data are represented as the mean ± SD (n=3). **P<0.01. *P<0.05 v.s. PBS.

**Figure S3 Expression level of LDL receptor on Hepalc1c7 cells.**

Expression level of LDL receptor was determined by flow cytometric analysis. Hepalc1c7 cells (1.0 × 10⁵ cells/well) were seeded in a 6-well plate, and then incubated for 24 hr. The cells were washed with PBS twice, and detached by the treatment with 0.05% trypsin-PBS. The detached cells were incubated with anti-murine LDL receptor antibody (1:100 dilution, Abcam, Tokyo, Japan) for 30 min at 4°C, followed by the incubation with a FITC labeled anti-armenian hamster IgG antibody (1:100 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 30 min at 4°C. Ten thousand cells per sample were analyzed using a FACSCalibur flow cytometer (BD Biosciences). The results were analyzed using Cell-Quest (BD Biosciences).