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LETTERS

Silencing of microRNAs *in vivo* with 'antagomirs'

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MicroRNAs (miRNAs) are an abundant class of non-coding RNAs that are believed to be important in many biological processes through regulation of gene expression[1–3]. The precise molecular function of miRNAs in mammals is largely unknown and a better understanding will require loss-of-function studies *in vivo*. Here we show that a novel class of chemically engineered oligonucleotides, termed 'antagomirs', are efficient and specific silencers of endogenous miRNAs in mice. Intravenous administration of antagomirs against miR-16, miR-122, miR-192 and miR-194 resulted in a marked reduction of corresponding miRNA levels in liver, lung, kidney, heart, intestine, fat, skin, bone marrow, muscle, ovaries and adrenals. The silencing of endogenous miRNAs by this novel method is specific, efficient and long-lasting. The biological significance of silencing miRNAs with the use of antagomirs was studied for miR-122, an abundant liver-specific miRNA. Gene expression and bioinformatic analysis of messenger RNA from antagomir-treated animals revealed that the 3′ untranslated regions of upregulated genes are strongly enriched in miR-122 recognition motifs, whereas downregulated genes are depleted in these motifs. Analysis of the functional annotation of downregulated genes specifically predicted that cholesterol biosynthesis genes would be affected by miR-122, and plasma cholesterol measurements showed reduced levels in antagomir-122-treated mice. Our findings show that antagomirs are powerful tools to silence specific miRNAs *in vivo* and may represent a therapeutic strategy for silencing miRNAs in disease.

Approaches to the study of miRNA function in mammals have focused on the overexpression or inhibition of miRNAs with antisense 2′-*O*-methyl (2′-*O*Me) oligoribonucleotides in cell lines as well as computational target predictions and validation by luciferase reporter assays[4–8]. However, functional studies in mice that lack specific miRNAs have yet to be reported. Further, because miRNAs may be important in human disease[6,9–12], approaches to interrupt miRNA function may have therapeutic utility. Small interfering double-stranded RNAs (siRNAs) engineered with certain 'drug-like' properties such as chemical modifications for stability and cholesterol conjugation for delivery have been shown to achieve therapeutic silencing of an endogenous gene *in vivo*[13]. To develop a pharmacological approach for silencing miRNAs *in vivo*, we designed chemically modified, cholesterol-conjugated single-stranded RNA analogues complementary to miRNAs, and have termed these oligonucleotides 'antagomirs'.

To explore the potential of these synthetic RNA analogues to silence endogenous miRNAs, we designed an antagomir, antagomir-122, selective for miR-122, an abundant, liver-specific miRNA. Antagomir-122 was synthesized starting from a hydroxyprolinol-linked cholesterol solid support[14] and 2′-*O*Me phosphoramidites. This compound was administered to mice by intravenous injection in a small volume (0.2 ml) at normal pressure. Administration of antagomir-122 resulted in a marked decrease in endogenous miR-122 levels as detected by northern blots (Fig. 1a). Administration of unmodified single-stranded RNA (anti-122) had no effect on levels of hepatic miR-122 (Fig. 1a), whereas injection of unconjugated, but chemically modified, single-stranded RNAs with a

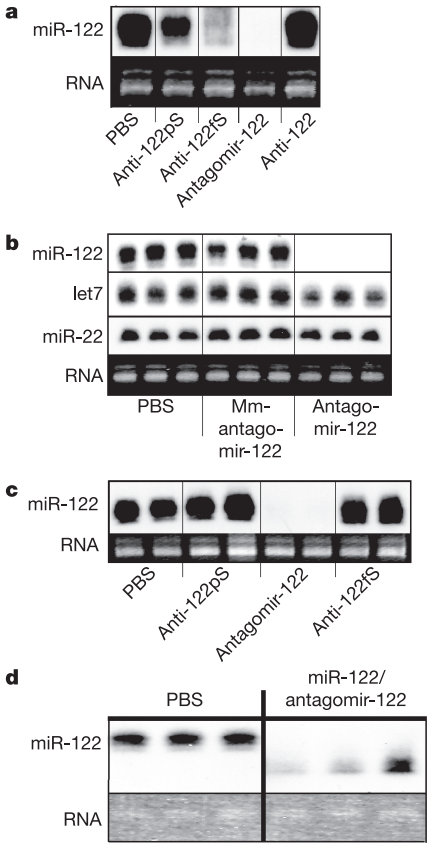

Figure 1 | Specific targeting of miR-122 in mouse liver by tail-vein injection of chemically modified single-stranded RNAs. a, Northern blots of total RNA (15 μg) isolated from mouse liver 24 h after injection of differently modified RNAs (three injections of 80 mg kg^{-1} d^{-1}) against miR-122. Samples were separated in 14% polyacrylamide gels in the absence (**a**, **b**) or presence (**c**) of 20% formamide. Ethidium bromide staining of tRNA is shown as a loading control. **d**, Mice were injected intravenously with PBS or a miR-122–antagomir-122 duplex (three injections of 80 mg kg^{-1} d^{-1}) and the livers were harvested 24 h after the last injection. Expression of miRNA-122 was analysed by northern blotting.

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partially modified (pS) or fully modified (fS) phosphorothioate backbone and 2′-*O*Me sugar modifications (anti-122pS and anti-122fS, respectively) led to an incomplete effect (Fig. 1a). The effects of antagomir-122 were found to be specific because animals injected with a control for antagomir-122 that harboured four mismatch mutations (mm-antagomir-122) had no effect on miR-122 levels (Fig. 1b). Furthermore, expression levels of let7 and miR-22 were unaffected in mice treated with antagomir-122 and mm-antagomir-122, indicating that silencing was miRNA-specific (Fig. 1b).

MicroRNA-122 is expressed at high levels in hepatocytes, with more than 50,000 copies per cell[15]. To examine whether the decrease in miR-122 after treatment with antagomir was due to degradation or to stoichiometric duplex formation between miR-122 and antagomir-122, with the resulting inability of the probe to detect miR-122 in a northern blot, we analysed total RNA from livers of mice treated with unconjugated anti-miR-122 oligonucleotides (anti-122fS and anti-122pS) or antagomir-122 under stringent denaturing conditions (Fig. 1c). No decrease in miR-122 levels could be detected with unconjugated anti-miR-122 RNA-treated livers, showing that the decrease in miR-122 levels observed under non-denaturing conditions (Fig. 1a) was simply due to the formation of miR-122–RNA duplexes. In contrast, miR-122 remained undetectable in livers of mice treated with antagomir-122 and was not lost during the RNA extraction procedure (Supplementary Figs 1 and 2). Further, we were able to detect specific miR-122 degradation products when the amount of miR-122 was increased by delivering preformed miR-122–antagomir-122 duplexes into the liver (Fig. 1d). These data indicate that the silencing of miRNA-122 in mice treated with antagomir-122 might have been due to degradation of the miRNA. The ability of antagomir-122 treatment to result in miR-122 degradation is probably due to the efficient delivery to hepatocytes and/or a consequence of changes in subcellular localization of antagomir–miR-122 complexes.

Several pharmacological properties for antagomirs were evaluated further, including dose–response, duration of action, and biodistribution. To determine the dose of antagomir-122 that can completely silence miR-122 we injected mice with a total dose of 80, 160 or 240 mg per kg body weight and analysed for miR-122 expression levels. The highest dose resulted in a complete loss of miR-122 signal (Supplementary Fig. 3a). We also tested the duration of silencing that could be achieved after the injection of antagomir-122. Levels of miR-122 were undetectable for as long as 23 days after injection, indicating that silencing of miRNAs using antagomirs is long lasting (Supplementary Fig. 3b). We next investigated the bioavailability and silencing activity of antagomirs in different tissues, using miR-16 as a target because it is expressed abundantly in all tissues. In mice treated with antagomir-16, miR-16 was efficiently silenced in all tissues tested except brain (Fig. 2a). Antagomir-16 did not seem to affect the expression of the 89-nucleotide precursor of miR-16 detected in bone marrow. The bioavailability of antagomir-16 was also assessed by northern blotting in the above-mentioned tissue samples. Consistent with the ability to silence miR-16 was our detection of significant levels of antagomir-16 in all tissues except brain (Fig. 2b). These data show that antagomirs achieve broad biodistribution and can efficiently silence miRNAs in most tissues *in vivo*.

Many miRNA genes have been found to be located close together and to be coordinately transcribed. These polycistronic miRNA genes result in long primary transcripts (pri-miRNAs) that are processed by multiple enzymes in the nucleus and cytoplasm to generate the mature miRNAs[16]. To investigate whether antagomirs targeting polycistronic miRNAs retain their target specificity with no effect on the expression of co-transcribed miRNAs, we injected mice with antagomirs targeting either miR-192 or miR-194 of the bicistronic cluster miR-192/194. Administration of antagomir-192 into mice resulted in the silencing of miR-192 in liver and kidney, with no effect on the expression levels of miR-194. The converse effects were seen with injection of antagomir-194 (Supplementary Fig. 4). These data show that antagomirs have the ability to differentially silence specific miRNAs that derive from the same primary transcript.

MicroRNAs can regulate the mRNA levels of their targets[17,18], and pharmacological silencing of miRNAs using antagomirs might therefore lead to the regulation of many mRNAs. To identify genes that are regulated by miR-122 we performed gene-expression analysis with Affymetrix arrays in livers from mice treated with antagomir-122 and mm-antagomir-122. In all, 363 transcripts were upregulated (at least 1.4-fold) and 305 transcripts were downregulated (at least 1.4-fold) in antagomir-122-treated mice compared with controls (Supplementary Table 1). The regulation of genes that were upregulated was confirmed by reverse-transcriptase-mediated polymerase chain reaction (RT–PCR; Fig. 3a). These included those members of gene families that are usually repressed in hepatocytes, including those

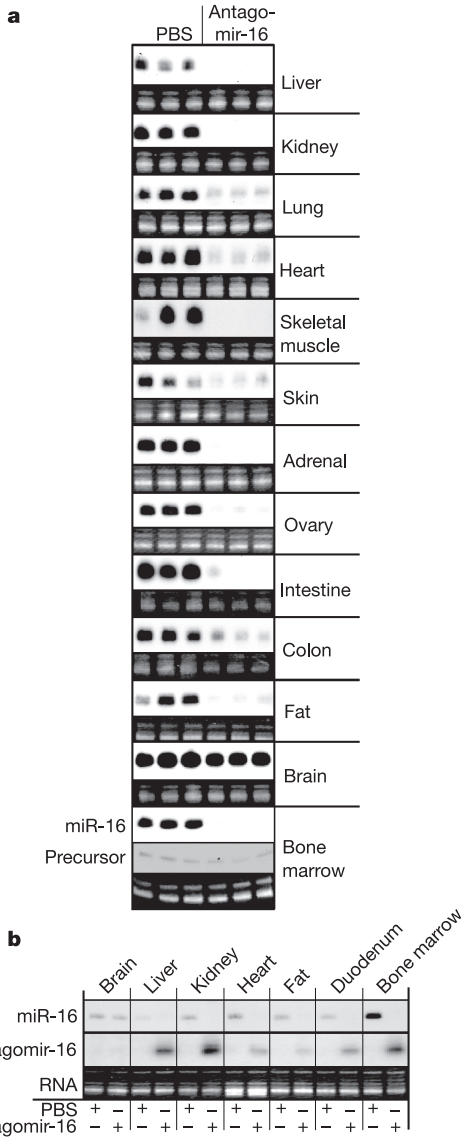


Figure 2 | Antagomirs target microRNA expression in multiple tissues. **a**, Northern blots of total RNA (10–30 μg) isolated from different mouse tissues 24 h after injection of antagomir-16 ($n = 3$). The precursor miRNA was visible on northern blots of bone marrow. Membranes were probed for miR-16. **b**, Total RNA from three mice as shown in **a** were pooled for the detection of miR-16 and the injected antagomir-16. Ethidium bromide staining of tRNA is shown as a loading control.

encoding aldolase-A (aldo-A), N-Myc downstream regulated gene 3 (Ndrg3) and IQ-motif-containing GTPase-activating protein 1 (Iqgap1). Therefore, miR-122 could contribute to the maintenance of the adult liver phenotype, as previously suggested for two other tissue-specific miRNAs[17]. To assess the motif contents of significantly upregulated and downregulated genes further, we analysed the 3′ untranslated region (UTR) sequences of 9,554 mRNAs that have annotated 3′ UTRs. Of these, 142 mRNAs were statistically significantly upregulated with at least a 1.4-fold change. Figure 3b shows the percentage of genes that had at least one miR-122 recognition motif CACTCC, corresponding to nucleotides 2–7 of miR-122—the core 'nucleus' sequence[19] (also referred to as the 'seed' sequence[20]). We observed a highly significant 2.6-fold increase in the probability of having at least one miR-122 nucleus in the 3′ UTR of upregulated transcripts in comparison with genes with no change in mRNA levels (Fig. 3b). Many of the miR-122 nucleus sequences in upregulated genes had not previously been predicted[19–21], indicating that the number of direct miRNA targets might be significantly larger than previously estimated.

For experimental validation of the link between repression and the presence of miR-122 nucleus matches within the 3′ UTR, we cloned the 3′ UTR of five genes upregulated by antagomir-122 and containing a miR-122 nucleus sequence into a luciferase reporter system. When co-transfected with miR-122, all reporters exhibited significant repression relative to co-transfections with control miRNA, showing that miR-122 binding to its nucleus contributes directly to mRNA repression (Fig. 3c). Of 108 transcripts that were significantly downregulated, we observed that the probability of harbouring a

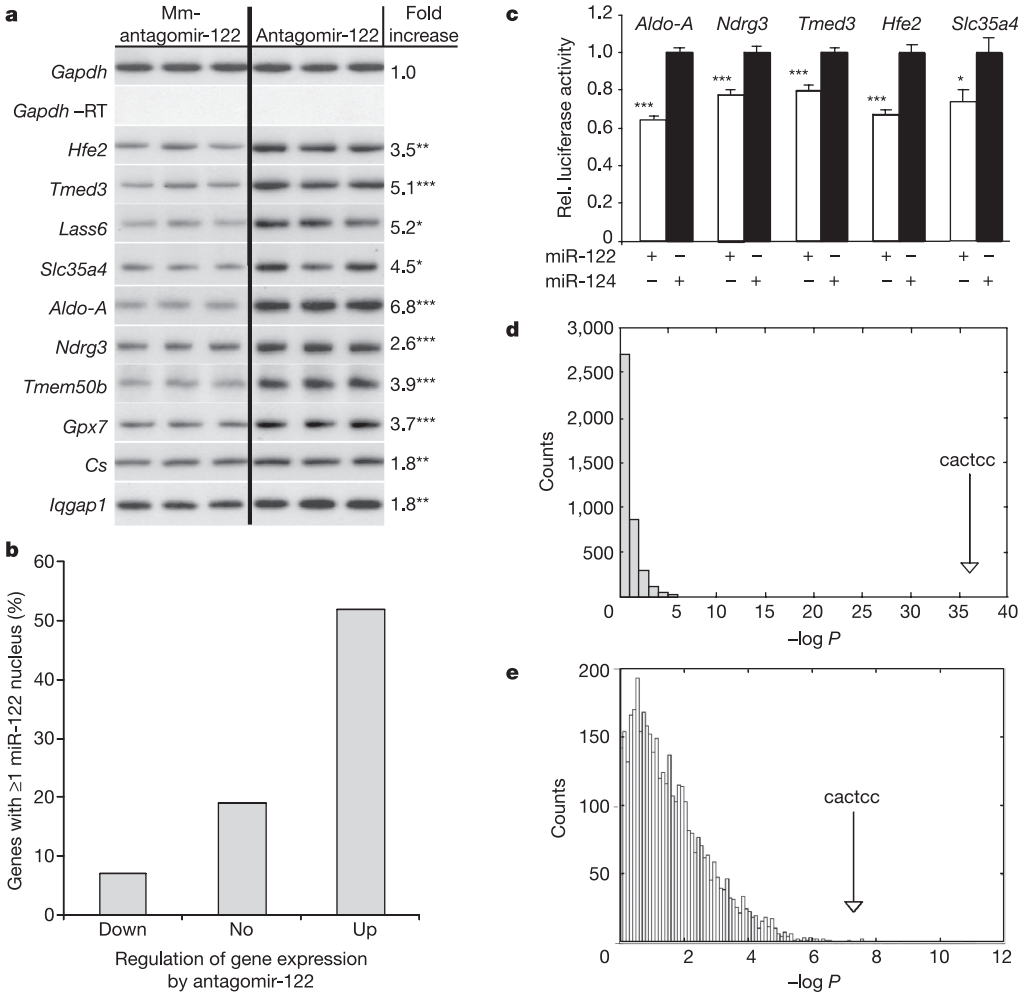
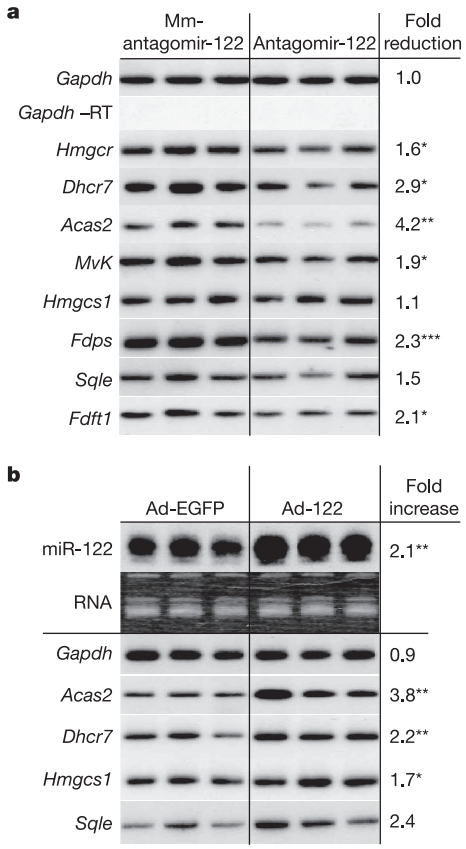


Figure 3 | Positive and negative regulation of gene expression by miRNA-122. **a**, Steady-state mRNA levels of genes in livers of mice treated with mm-antagomir-122 or antagomir-122. Expression was measured by RT–PCR. Each lane indicates an individual animal. Gene symbols are shown in accordance with the International Standardized Nomenclature (www.informatics.jax.org/mgihome/nomen/gene.shtml). Fold increase indicates the ratio of expression levels of the means of mice treated with antagomir-122 and mm-antagomir-122. The glyceraldehyde-3-phosphate dehydrogenase gene (*Gapdh*) was used as a loading control. **b**, Abundance of the miR-122 nucleus in differentially expressed genes. The plot shows the percentage of genes with at least one miR-122 recognition motif present in their 3′ UTR. The significance of the lower (higher) percentage of downregulated (upregulated) genes with a miR-122 nucleus was assessed by 1,000 random poolings of the same number of genes for each class from the total set of transcripts. The result for downregulated genes was significant at three standard deviations ($P = 0.001$). The result for upregulated genes was significant at more than eight standard deviations. **c**, Micro-RNA-directed repression of *Renilla* luciferase reporter genes bearing 3′ UTR segments from genes identified from microarray expression analysis of antagomir-122-treated mice after co-transfection into HEK-293 cells with the indicated miRNA (si-122 or si-124). Data are from three independent experiments and are shown as means ± s.e.m., with $n = 6$. **d**, For each of the 4,096 possible hexamer RNA motifs and each transcript, the number of non-overlapping occurrences divided by the length of the 3′ UTR was recorded. For each motif, a non-parametric test (the one-tailed Wilcoxon rank sum test) was applied to these distributions in upregulated versus no-change transcripts. Shown is the histogram of the negative natural logarithm of all 4,096 *P* values. **e**, Analogously to **d**, comparison between transcripts with no significant change in expression and downregulated transcripts. Asterisk, $P < 0.05$; two asterisks, $P < 0.01$; three asterisks $P < 0.001$.

miR-122 nucleus was decreased by almost the same factor, 2.7-fold (Fig. 3b). To analyse further whether the over-representation and under-representation of miR-122 nucleus sequences are specific, we analysed the abundance of all 4,096 possible hexamer motifs across downregulated, upregulated and unchanged transcripts. When we compared upregulated with unchanged genes, the miR-122 nucleus sequence was the most significantly over-represented hexamer (Fig. 3d). Notably, the miR-122 nucleus was within the top 1% of under-represented motifs for downregulated transcripts (Fig. 3e), indicating a possible evolutionary tendency of downregulated genes to lack binding sites for miR-122. These results indicate that upregulated mRNAs are directly targeted and repressed by miR-122, but also that a significant number of downregulated genes are likely to be activated by miR-122.



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	Mm-antagomir-122		Antagomir-122		*n*=5	Fold
	Mean	s.e.m.	Mean	s.e.m.	*P*	reduction
Age (weeks)	7.7	0.0	7.7	0.0	1.000	
Body weight (g)	16.3	0.3	16.2	0.3	0.792	
Glucose (mg dl^{-1})	138.0	7.0	152.0	13.0	0.429	
FFA (mM)	0.42	0.06	0.37	0.07	0.586	
Bile acids (μM)	10.9	3.2	12.2	4.2	0.822	
Triglycerides (mg dl^{-1})	31.9	5.3	29.7	4.4	0.754	
Cholesterol (mg dl^{-1})	39.7	3.3	22.1	1.9	0.003	–0.44
ALT (U l^{-1})	23.0	7.0	26.0	4.0	0.734	

Figure 4 | MicroRNA-122 regulates the expression of genes involved in cholesterol biosynthesis. a, RT–PCR analysis of cholesterol biosynthesis genes identified in Affymetrix gene-expression analysis in livers of mice treated with antagomir-122 or mm-antagomir-122. Fold reduction indicates the ratio of expression levels of the means of mice treated with mm-antagomir-122 and antagomir-122. The glyceraldehyde-3-phosphate dehydrogenase gene (*Gapdh*) was used as a loading control. Gene abbreviations: *Hmgcr*, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; *Dhcr7*, 7-dehydrocholesterol reductase; *Acas2*, acetyl-coenzyme A synthetase 2 (ADP forming); *Mvk*, mevalonate kinase; *Hmgcs1*, 3-hydroxy-3-methylglutaryl-coenzyme A synthase 1; *Fdps*, farnesyl diphospate synthetase; *Sqle*, squalene epoxidase; *Fdft1*, farnesyl diphosphate farnesyl transferase 1. Each lane represents an individual animal. **b**, RT–PCR analysis of cholesterol biosynthesis genes in animals 6 days after injection of Ad-EGFP or Ad-122. The upper row shows a northern blot of liver RNA for miR-122. **c**, Metabolic measurements in mice treated with antagomir-122 and mm-antagomir-122 control. FFA, non-esterified fatty acid. Asterisk, $P < 0.05$; two asterisks, $P < 0.01$; three asterisks, $P < 0.001$.

To assess the phenotype of altered gene regulation by miR-122, we analysed the annotation of regulated genes for enrichment in Gene Ontology categories. The top-ranking functional category was 'cholesterol biosynthesis' ($P = 1.6 \times 10^{-11}$) and was found for gene transcripts downregulated by antagomir-122. The expression of at least 11 genes involved in cholesterol biosynthesis was decreased between 1.4-fold and 2.3-fold in antagomir-122-treated mice (Supplementary Table 1); some of these were confirmed by RT–PCR (Fig. 4a). Mice injected with an adenovirus expressing miR-122 (Ad-122) increased the expression of some of these genes (Fig. 4b). One of the gene transcripts downregulated by treatment with antagomir-122 was 3-hydroxy-3-methylglutaryl-CoA-reductase (Hmgcr), a rate-limiting enzyme of endogenous cholesterol biosynthesis. We measured the enzymatic activity of Hmgcr in liver extracts and found a roughly 45% decrease in activity in antagomir-122-treated mice compared with mm-antagomir-122-treated mice (9.7 ± 1.0 and 17.2 ± 2.3 pmol per mg of microsomal protein per minute, respectively; $n = 4$, $P = 0.02$). Consistent with this effect was the observation that plasma cholesterol levels were decreased more than 40% in treated animals whereas there was no detectable effect on plasma non-esterified fatty acids, triglyceride, bile acid and glucose levels (Fig. 4c). No decrease in cholesterol was observed with antagomir-16, antagomir-192 and antagomir-194, showing that the effects of antagomir-122 are sequence-specific and unrelated to the use of a cholesterol-conjugated oligonucleotide itself. Decreased cholesterol levels in antagomir-122-treated mice lasted for at least 2 weeks (data not shown). Antagomir-122 was well tolerated even during the course of the prolonged treatment; no alterations in body weight or serum markers of liver toxicity (alanine aminotransferase (ALT) levels) were detected. Together, these data indicate that miR-122 participates in regulation of the cholesterol biosynthetic pathway and that silencing of a miRNA can be achieved without apparent toxicities.

The discovery of miRNAs is likely to change our understanding of gene expression fundamentally, yet almost nothing is known of their function in mammalian systems *in vivo*. Our data show that antagomirs can effectively silence miRNAs *in vivo*, and that antagomirs can enable the study of gene regulation *in vivo* by a tissue-specific miRNA, miR-122. In addition to many upregulated genes, silencing of miR-122 also led to a decrease in a significant number of genes. The mechanism by which miRNAs can activate gene expression is currently unknown but may be the result of a direct effect (chromatin remodelling) or an indirect effect (suppression of a transcriptional repressor). Notwithstanding the large number of genes regulated by antagomir treatment, it is striking, given the extent and duration of miR-122 silencing, that the phenotype of antagomir-122-treated mice was otherwise modest. Further studies are needed to explore changes at the protein level as a result of silencing multiple miRNAs as well as potential effects under stress conditions or in disease models, but our data support the model that some miRNAs might serve more as a 'rheostat' than as an 'on–off switch', to fine-tune gene expression. Our novel pharmacological approach to silence miRNAs specifically will allow the rapid generation of mice lacking specific miRNAs or combinations of miRNAs for further functional studies. Finally, because it has been shown that miRNAs are involved in cancer[9–12], cell growth and differentiation[4,5,22], insulin secretion[6] and viral infection[23], silencing of miRNAs with antagomirs could become a therapeutic strategy for diseases[24] such as cancer, hepatitis and diabetes, and others almost certain to be discovered, in which miRNAs participate in disease aetiology.

METHODS

Synthesis of antagomirs. The single-stranded RNAs and modified RNA analogues used in this study consisted of a 21–23-nucleotide length with modifications as specified: anti-122, 5′-ACAAACACCAUUGUCACACUCCA-3′; anti-122pS, 5′-$a_sc_saaacaccauugucacac_su_sc_sc_sa$-3′; anti-122fS, 5′-$a_sc_sa_sa_sa_sc_sa_sc_sc_sa_su_s-u_sg_su_sc_sa_sc_sa_sc_su_sc_sc_sa$-3′; antagomir-122, 5′-$a_sc_saaacaccauugucacacu_sc_sc_sa_s$-Chol-3′; mm-antagomir-122, 5′-$a_sc_sacacaacacugucacauu_sc_sc_sa_s$-Chol-3′; antagomir-16, 5′-$c_sg_sccaauauuuacgugcug_sc_su_sa_s$-Chol-3′; antagomir-192, 5′-$g_s-g_scugucaauucauaggu_sc_sa_sg_s$-Chol-3′; antagomir-194, 5′-$u_sc_scacauggaguug cuguu_sa_sc_sa_s$-Chol-3′. The lower case letters represent 2′-*O*Me-modified nucleotides; subscript 's' represents a phosphorothioate linkage; 'Chol' represents cholesterol linked through a hydroxyprolinol linkage[14]; anti-122pS is anti-miR-122 RNA with partial phosphorothioate backbone and anti-122fS is anti-miR-122 RNA with full phosphorothioate backbone. Details of the synthesis are given in the Supplementary Methods.

Animals. All animal models were maintained in a C57BL/6J background on a 12-h light/dark cycle in a pathogen-free animal facility at Rockefeller University. Six-week-old mice received, on one to three consecutive days, tail-vein injections of saline or different RNAs (as indicated). RNAs were administered at doses of 80 mg per kg body weight in 0.2 ml per injection. Measurements of miRNA levels in tissues were performed 24 h after the last injection unless indicated otherwise. Tissues were harvested, snap-frozen and stored at −80 °C.

Generation of recombinant adenovirus. The recombinant adenovirus used to express miR-122 (Ad-122) was generated by PCR, amplifying a 344-base-pair miRNA precursor sequence with primers 5′-AGTCAGATGTACAGTTATAAG CACAAGAGGACCAG-3′ and 5′-TTATTCAAGATCCCGGGGCTCTTCC-3′. The fragment was cloned into vector Ad5CMV-KnpA. Ad-EGFP (ViraQuest) was used as a control. Mice were infected with 5×10^9 plaque-forming units per mouse by injection into the tail vein.

Gene-expression analysis. Total RNA from liver of mice treated with antagomirs was isolated three days after the initiation of treatment. RNA was pooled from three animals for each group. The generation and analysis of Affymetrix microarray data are described in the Supplementary Methods.

Northern blotting analysis. Total RNA was isolated with the Trizol reagent (Invitrogen) and precipitation with ethanol. RNA was separated at 45 mA on 14% polyacrylamide gels containing 8 M urea and 20% formamide in a Protean II xi vertical electrophoresis cell (Bio-Rad). Samples were transferred to Hybond-N^+ nylon membranes (Amersham) in a Trans-Blot electrophoretic transfer cell (Bio-Rad) for 2 h at 1 A. DNA antisense probes were designed as described in the 'microRNA registry' (http://www.sanger.ac.uk) and 20 pmol of each was labelled with T4 polynucleotide kinase (New England Biolabs) and 30 μCi of [γ-^{32}P]ATP (3,000 Ci mmol^{-1}; NEN Life Science). Hybridizations were performed at 50 °C for 16 h in a 20 mM sodium phosphate buffer pH 7.2 containing 7% SDS, 0.75 M NaCl, 75 mM sodium citrate, 0.02% albumin, 0.02% polyvinylpyrrolidone, 0.02% Ficoll 400 and 0.1 mg ml^{-1} sonicated salmon-sperm DNA.

RT–PCR. Extraction of total RNA, synthesis of cDNA, and PCR were performed as described in the Supplementary Methods.

Assay of luciferase activity. Mouse full-length 3′ UTR sequences were PCR-amplified with specific primers and cloned downstream of the stop codon in pRL-TK (Promega). HEK-293 cells were cultured in 24-well plates and each well was transfected with 50 ng of the respective pRL-TK 3′ UTR contructs (Rr-luc), 50 ng of pGL3 control vector (Pp-luc) (Promega) and 200 ng of double-stranded siRNA (Dharmacon). Cells were harvested and assayed 24–30 h after transfection. Results were normalized to the Pp-luc control and are expressed relative to the average value of the control miRNA (si-124).

3′ UTR sequences and mapping of array probes to transcripts. We extracted mouse 3′ UTRs with the use of the Refseq data set (ftp://ftp.ncbi.nih.gov/refseq/)[25]. A total of 17,264 3′ UTR sequences at least 30 nucleotides in length were obtained. Affymetrix probe identifiers were assigned to the Refseq transcripts by using a mapping provided by Ensembl (http://www.ensembl.org/Multi/martview). When more than one probe identifier mapped to a transcript, we insisted that the Affymetrix significance call be consistent for all probes. Transcripts were discarded otherwise. The fold change assigned to a transcript was the average of all probes that mapped to the transcript. Finally, a cut-off of 0.5 in the $\log_2$ of fold changes was applied.

Gene ontology analysis. The analysis is described in the Supplementary Methods.

Hmgcr activity assay. Hepatic microsomal Hmgcr activity was assayed by a method modified from a previously published procedure and described in detail in the Supplementary Methods.

Statistical analysis. Results are given as means ± s.e.m. Statistical analyses were performed with Student's *t*-test, and the null hypothesis was rejected at the 0.05 level.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Information Gene expression data have been deposited at GEO-NCBI under accession number GSE3425. Reprints and permissions information is available at npg.nature.com/reprintsandpermissions. The authors declare competing financial interests: details accompany the paper at www.nature.com/nature. Correspondence and requests for materials should be addressed to M.S. (stoffel@rockefeller.edu).