读书报告

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2016-04-09

目录

（一）miRNA与脂代谢

miRNA是一类长度约22个核苷酸的内源性非编码单链RNA，在细胞增殖、分化、凋亡和肿瘤发生等基本生物过程中起着不同作用，是基因表达的重要调控因子。（Bartel 2004)

（一）miRNA与脂代谢

miRNA**广泛存在**于植物和动物等生物体中，具有一定的***保守性***，有一些mRNA 广泛存在于各种生物体或组织，而有一些miRNA存在于某种特定生物体或组织。通常一种miRNA可以调控成百种mRNA，一种mRNA可以由成百种miRNA调控，这样miRNA在生物体内构成一个极其复杂的网络调控系统，从而发挥着重要作用。（Brennecke 等，2005；Krek 等，2005；Doench 等，2004）

（一）miRNA与脂代谢

关于脂代谢，miRNA主要是通过抑制脂代谢相关基因的翻译来影响脂肪、胆固醇的合成、转运以及利用，因此miRNA可作为脂代谢调节的潜在媒介。

miR-33　　miR-122　　miR-27

（一）miRNA与脂代谢------miR-33与脂代谢

ABCA1基因具有3个高度保守的miR-33结合靶位点，miR-33能够靶向抑制ABCA1基因的表达。

抑制miR-33a和miR-33b生成也导致肝脏ABCA1基因表达量增加以及血浆HDL的持续性增加，且还导致miR-33的其他**脂肪酸氧化相关靶基因**［如肉碱氧位甲基转移酶（CROT）、肉碱棕榈酰基转移酶1A（CPT1A）、羟酰辅酶A脱氢酶（HADHB）和AMP激活的蛋白激酶α1亚基（PRKAA1）］的表达量增加；

（一）miRNA与脂代谢-------miR-33与脂代谢

以及***脂肪酸合成基因***［如SREBF1、FAS、ATP柠檬酸裂解酶（ACLY）和乙酰辅酶 A 羧化酶 α （ACACA）基因］的表达量降低，最终使得血浆极低密度脂蛋白和甘油三酯水平显著降低。（Najafi-shoushtar 等，2010；Rayne 等，2010；Rayne 等，2011；Rayne 等，2012）

（一）miRNA与脂代谢------miR-122与脂代谢

miR-122占成年小鼠肝脏miRNA的70%，且在物种间高度保守，它是由一种非编码的基因hcr的转录本加工而来，这种基因在物种间也较保守。**肝脏中miR-122的表达对脂代谢具有重要的调节作用**，miR-122过表达或抑制都能导致胆固醇以及脂肪酸合成相应的变化。（Tsai 等，2012；Chang 等，2004）

（一）miRNA与脂代谢-------miR-27与脂代谢

在细胞中miR-27过表达能够降低LPL基因的表达量，并抑制甘油三酯的积累以及脂肪生成基因的表达。miR-27a和miR-27b可以抑制细胞中**脂代谢相关基因**的表达，如PPARγ、视黄醛X受体α（RXRα）、脂联素、CD36、FAS、FABP4、葡萄糖转运蛋白4（GLUT4）和SREBP-1c等基因。(Kim 等，2010；Karbiener 等，2009)

（一）miRNA与脂代谢-------其它miRNA与脂代谢

有研究表示**miR-335**在脂质加工过程中表达上调，且在肥胖小鼠的肝脏和脂肪组织中高度表达。关于**miR-378**的研究表明，它可以增强脂肪酸代谢相关基因的表达，如FABP4、FAS和SCD1等基因。**miR-21**在肝功能以及胆固醇调节方面也起着一定的作用，不饱和脂肪酸能够上调miR-21的表达，且其在高脂饮食的大鼠以及人类的肥胖个体肝脏组织中表达量较高。Nakanish 等，2009；Gerin 等，2010；Vinciguerra 等，2009）

（二）miRNA功能研究

miRNA mimic----------miRNA模拟物
miRNA inhibitor---------miRNA抑制物
miRNA agomir----------miRNA激动剂
miRNA antagomir-------miRNA 拮抗剂

miRNA mimic是miRNA模拟物，化学合成的成熟miRNA双链，即用型；

miRNA inhibitor是miRNA抑制物，化学修饰的成熟miRNA互补单链，即用型；

miRNA agomir是特殊化学修饰的miRNA 激动剂，适用于细胞实验、动物实验，即用型；

miRNA antagomir是特殊化学修饰的miRNA 拮抗剂，适用于细胞实验、动物实验，即用型。

（二）miRNA功能研究

miRNA agomir是经过特殊化学修饰的miRNA激动剂，通过模拟miRNA进入miRISC复合物来调节靶mRNA的表达而发挥作用。

与普通的miRNA mimics相比，agomir在动物体内具有更高的稳定性和miRNA促进效果。且能克服体内细胞膜、组织等障碍富集于靶细胞。在动物实验中可以用全身或局部注射、吸入、喂药等方法进行给药，作用效果持续时间可长达6周。

（二）miRNA功能研究

miRNA antagomir是经过特殊化学修饰的miRNA拮抗剂，通过与体内的成熟miRNA强竞争性结合，阻止miRNA与其靶基因mRNA的互补配对，抑制miRNA发挥作用。

与普通抑制剂相比， miRNA antagomir在动物体内外具有更高的稳定性和抑制效果，且能克服体内细胞膜、组织等障碍富集于靶细胞。antagomir在细胞实验中不需要转染试剂，从而避免了转染试剂包装过程的复杂步骤及其对实验的影响。 在动物实验中可用全身或局部注射、吸入、喂药等方法进行给药，作用效果持续时间可长达6周。

（二）miRNA功能研究

antagomir 和agomir 具有如下优点：

1、与普通miRNA inhibitor和mimics相比，antagomir 和agomir与细胞膜亲和力更高，细胞转染实验转染试剂用量显著减少。

2、特别适合动物体内干扰实验.并且在体内实验中具有更高的稳定性和抑制效果，可以采用全身注射或局部注射等多种方式给药，操作简便。

3、可富集于靶细胞，实现高效的特异性稳定干扰

4、抑制持续时间长,至少达到一周时间,干扰效果最长可持续5-6周。

（三）miRNA文章


miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting
C Esau，S Davis，SF Murray，... 《Cell Metabolism》 - 2006 被引量: 1557
Current understanding of microRNA (miRNA) biology is limited, and antisense oligonucleotide () inhibition of miRNAs is a powerful technique for their fu...
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3. miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting
作者: Esau, C; Davis, S; Murray, SF; 等.
CELL METABOLISM 卷: 3 期: 2 页: 87-98 出版年: FEB 2006
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[HTML] miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting
sciencedirect.com 中的 [HTML]
C Esau, S Davis, SF Murray, XX Yu, SK Pandey... - Cell metabolism, 2006 - Elsevier
Current understanding of microRNA (miRNA) biology is limited, and antisense oligonucleotide (ASO) inhibition of miRNAs is a powerful technique for their functionalization. To uncover the role of the liver-specific miR-122 in the adult liver, we ...
被引用次数：1387 相关文章 所有 15 个版本 引用 保存

Cell Metabolism

Volume 3, Issue 2, February 2006, Pages 87–98

Article

miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting

Christine Esau[1], Scott Davis[1], Susan F. Murray[1], Xing Xian Yu[1], Sanjay K. Pandey[1], Michael Pear[1], Lynnetta Watts[1], Sheri L. Booten[1], Mark Graham[1], Robert McKay[1], Amuthakannan Subramaniam[1], Stephanie Propp[1], Bridget A. Lollo[1], Susan Freier[1], C. Frank Bennett[1], Sanjay Bhanot[1], Brett P. Monia[1]

Here we report that inhibition of miR-122 in both normal and high-fat fed mice with **a 2'-O-methoxyethyl (2'-MOE) phosphorothioatemodified antisense oligonucleotide** (ASO) for over 5 weeks was well tolerated and was associated with a significant reduction in hepatic steatosis and plasma cholesterol levels. These effects were accompanied by a reduction in hepatic sterol and fattyacid synthesis rates and stimulation of hepatic fatty-acid oxidation.The results suggest that miR-122 may be a therapeutic target for metabolic and cardiovascular diseases.

Experimental procedures

Oligonucleotide synthesis and sequence

Isolation and transfection of primary mouse hepatocytes and AML12

Animal care and treatments

Histological analysis

Metabolic measurements

HPLC analysis of lipoproteins

RT-PCR analysis

Northern blotting

Western blotting

Microarray

Determination of sterol and fatty-acid synthesis and fatty-acid oxidation rate

结果与分析

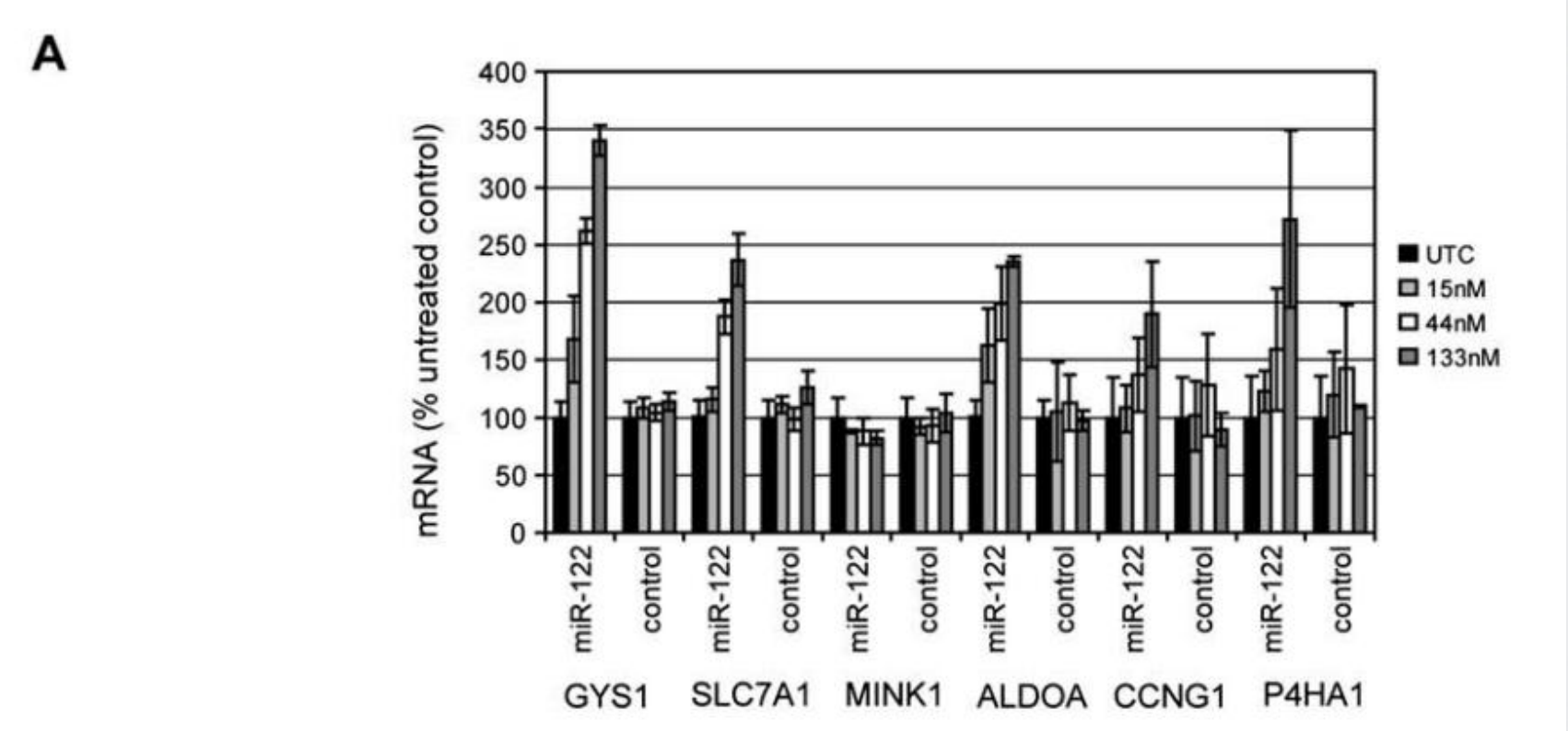


Figure 1. Identification of miR-122 target genes in vitro
A)TaqMan RT-PCR measuring mRNA of predicted miR-122 target genes after transfection of primary mouse hepatocytes with 20-MOEASO targetingmiR-122 or a control ASO

结果与分析

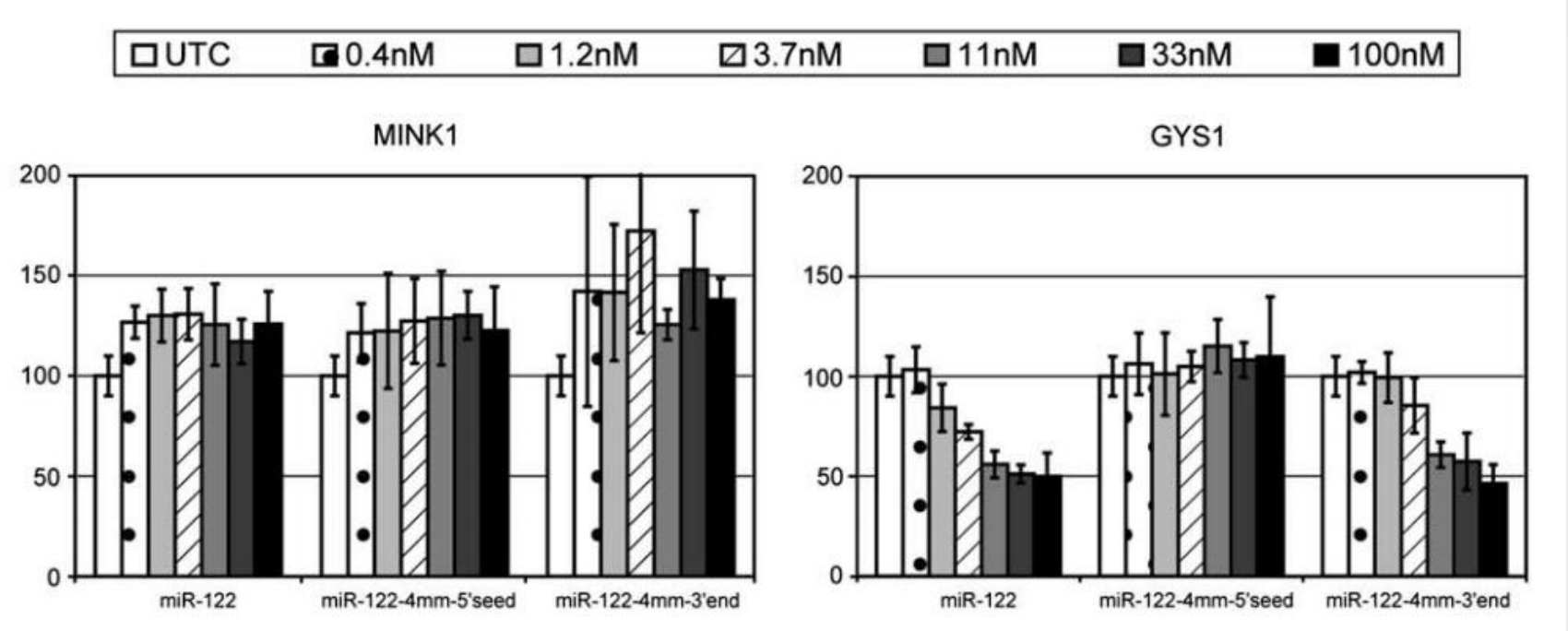
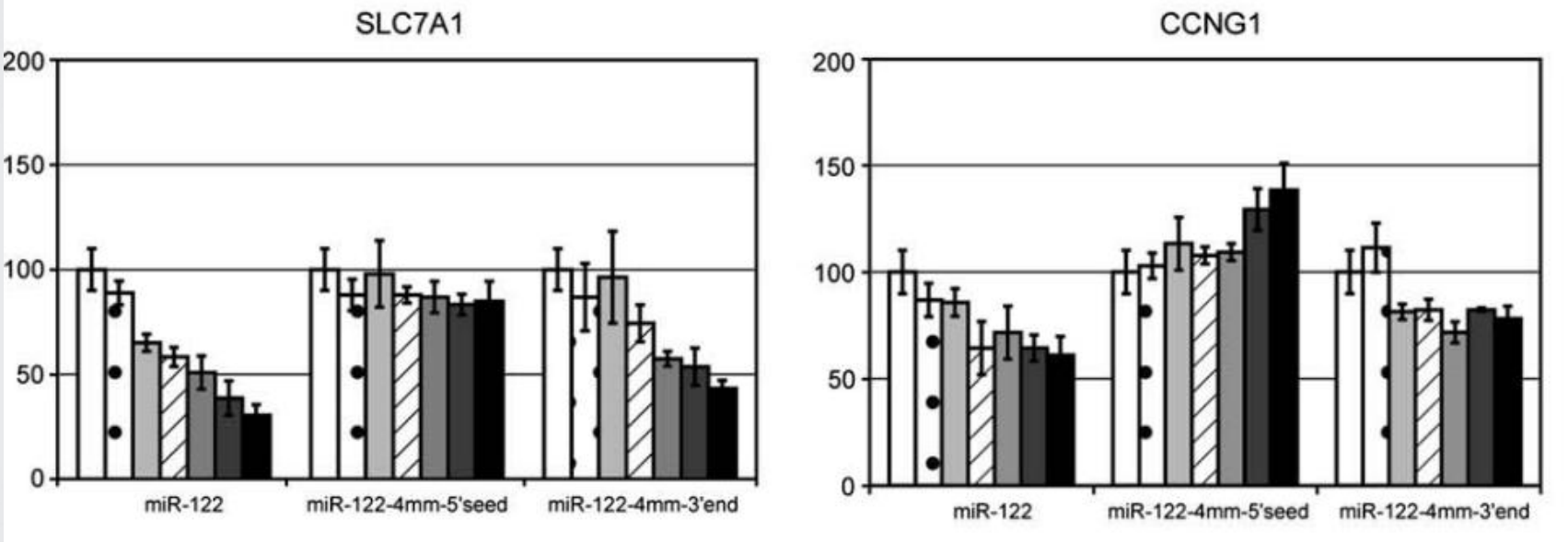


Figure 1. Identification of miR-122 target genes in vitro
B) TaqMan RT-PCR measuring mRNA of predicted miR-122 target genes after transfection of AML12 with miR-122 duplex RNA or miR-122 duplex RNA with four mismatches in the 50 seed region or the 30 half of the miRNA. Error bars represent standard deviation of triplicate samples.

结果与分析



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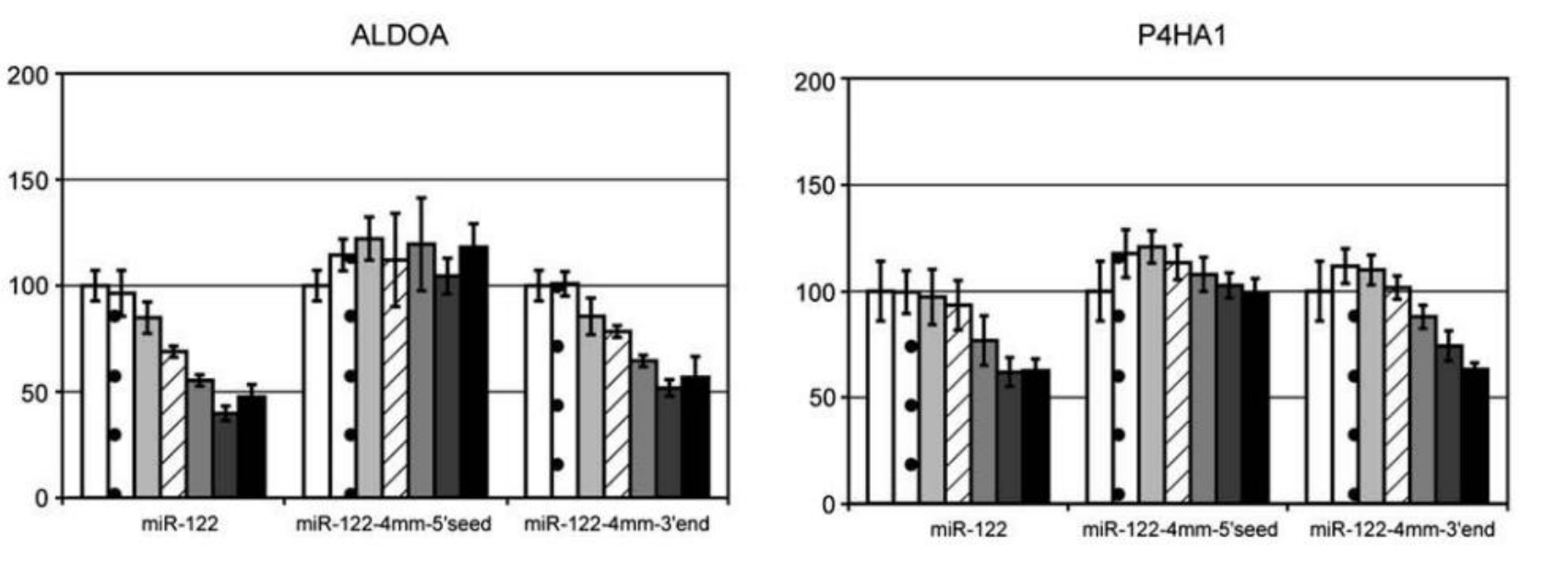
结果与分析



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B) TaqMan RT-PCR measuring mRNA of predicted miR-122 target genes after transfection of AML12 with miR-122 duplex RNA or miR-122 duplex RNA with four mismatches in the 50 seed region or the 30 half of the miRNA. Error bars represent standard deviation of triplicate samples.

举例：

Tab. 3 Prediction of miRNA target site

miRNA 名称 miRNA ID	位点 location	长度 length	杂交反应 hybridization	最低自由能 minimum free energy	分值 score
hsa-miR-33a	433 ~ 463	31	miRNA: 3′ cacuaCGUG—ACAC———CUUUGUAAc 5′ Target:5′ tattaGCACCTTTTGAAAGAAAGAAACATTa 3′	-8.5	147
hsa-miR-16	650 ~ 671	22	miRNA: 3′ gcGGUUAUAAAUGCACGACGAu 5′ Target:5′ ggTCGATTTTGTCGTGCTGCTg 3′	-22.9	168

结果与分析

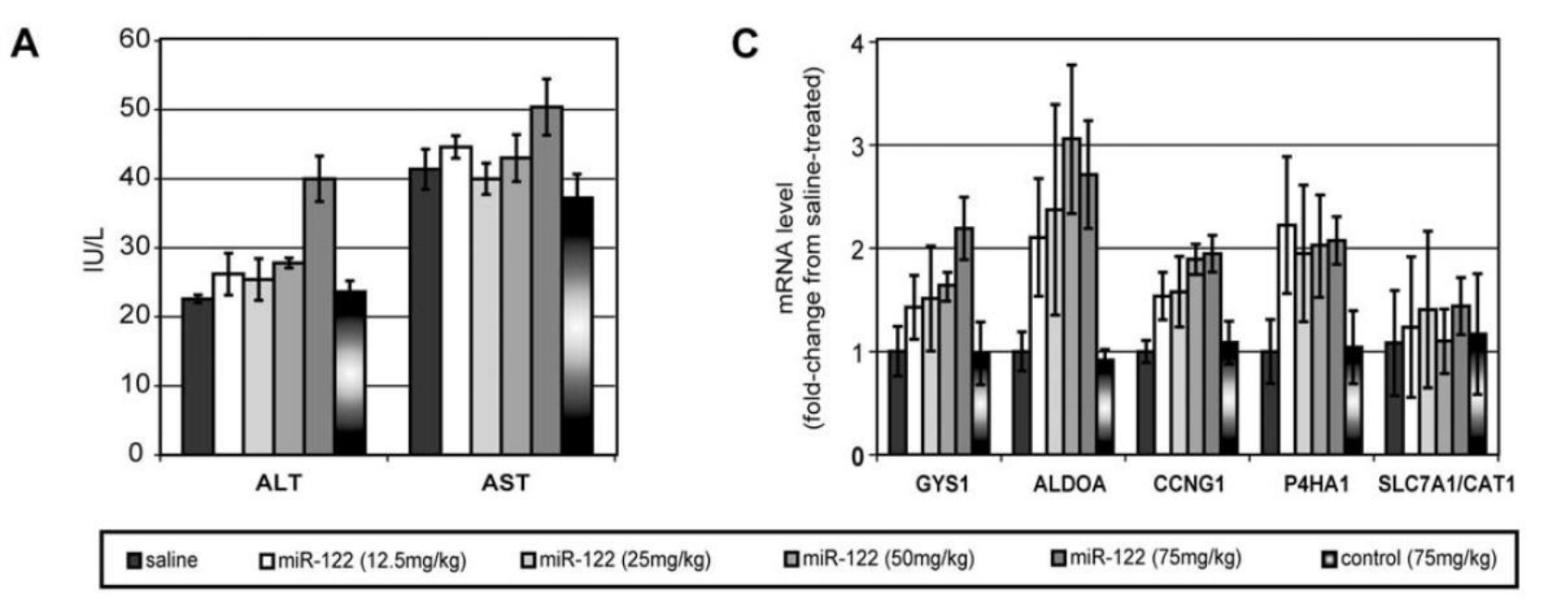


Figure 2. Inhibition of miR-122 in normal mice

Normal mice were treated i.p. with the indicated dose of miR-122 ASO or control ASO twice weekly for 4 weeks. n = 5.

A) Plasma transaminase levels measured at 4 weeks. Error bars = SEM.

C) TaqMan RT-PCR measuring levels of miR-122 target genes in liver RNA. Error bars = SD.

结果与分析

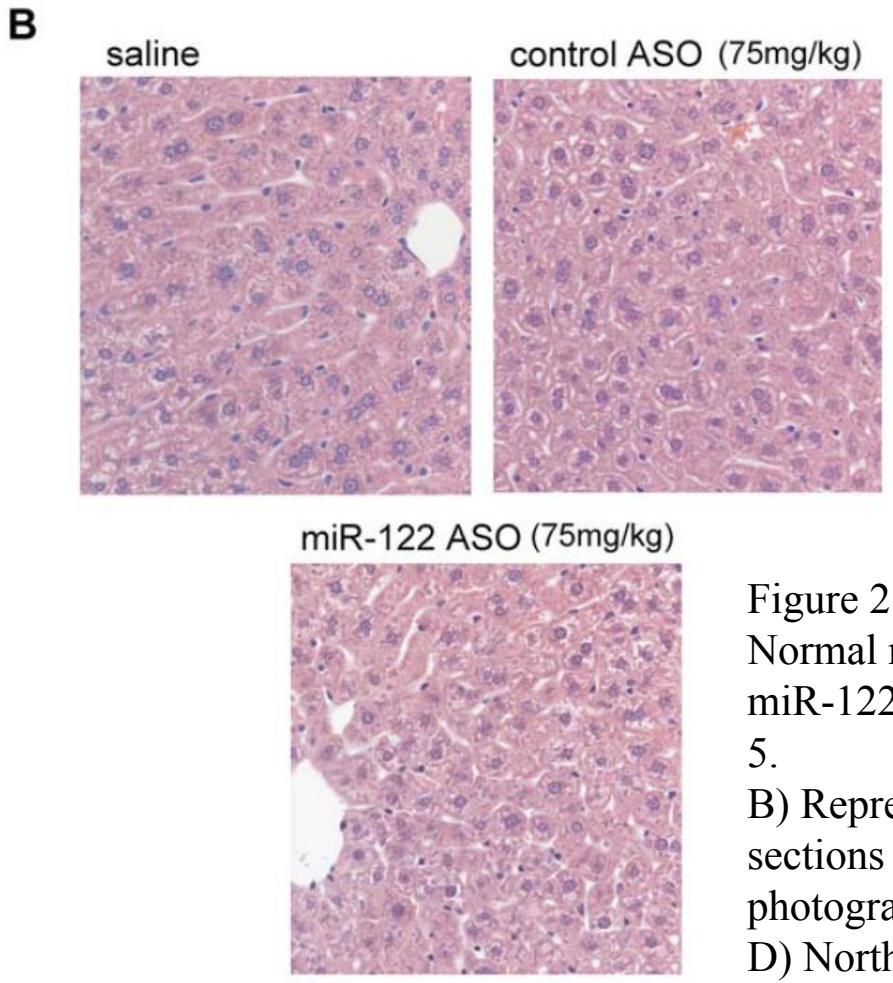


Figure 2. Inhibition of miR-122 in normal mice
Normal mice were treated i.p. with the indicated dose of miR-122 ASO or control ASO twice weekly for 4 weeks. n = 5.
B) Representative hematoxylin-and-eosin-stained liver sections from saline- or ASO-treated (75 mg/kg dose) mice, photographed at 403 magnification.
D) Northern blotting for miR-122 in liver RNA.

结果与分析

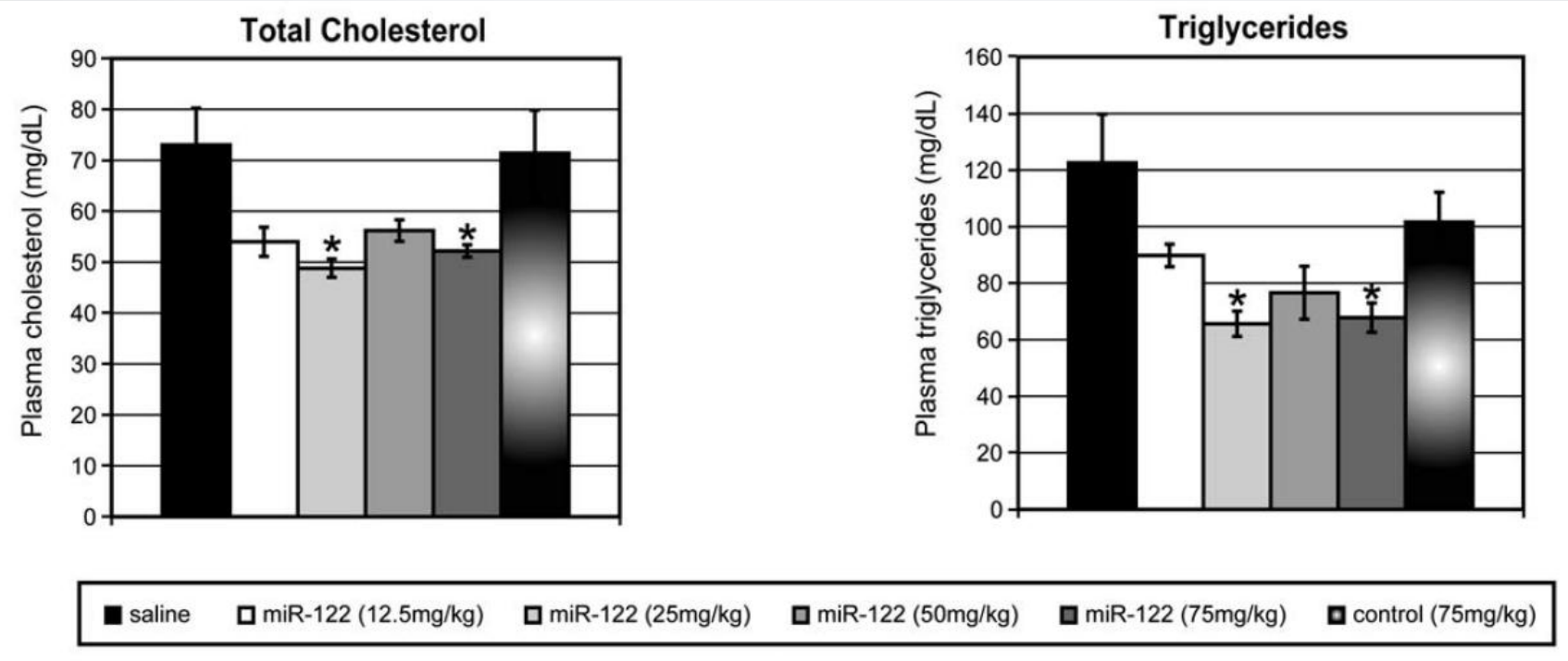
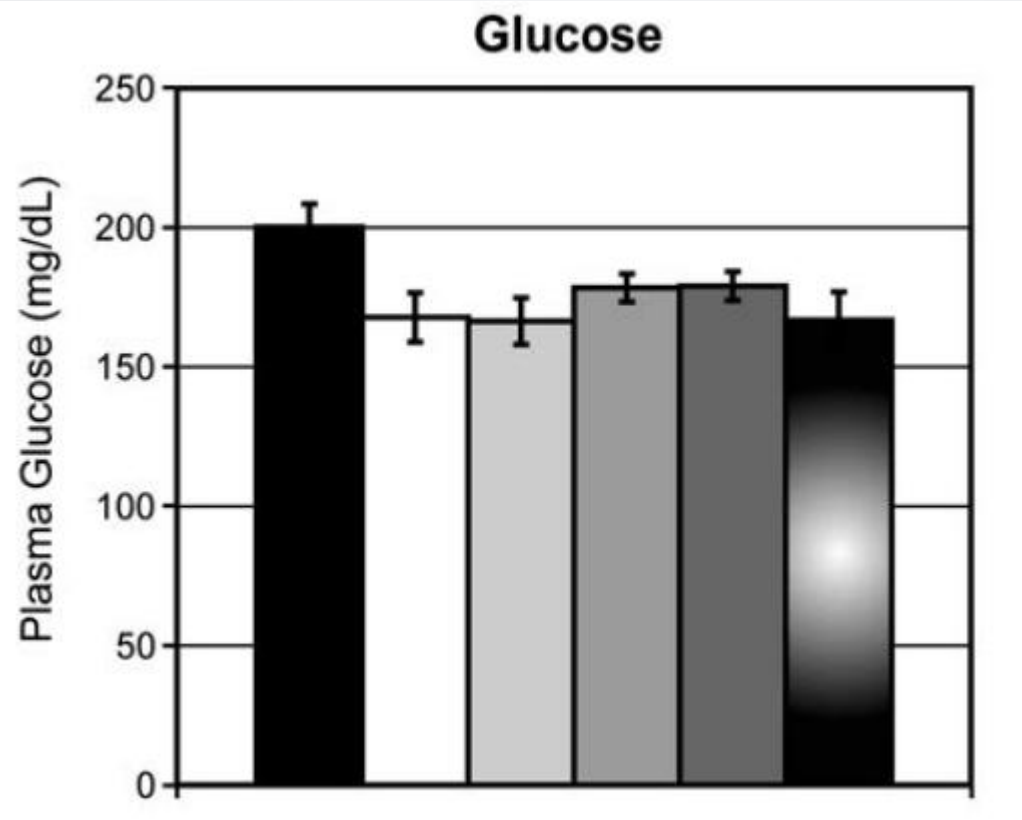


Figure 3. Plasma markers in normal mice after inhibition of miR-122
Plasma cholesterol, triglycerides, and glucose measured in normal mice that had been treated i.p. with the indicated dose of miR-122 or control ASO twice weekly for 4 weeks. n = 5. Error bars = SEM. *p < 0.05.

结果与分析



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结果与分析

To help elucidate the mechanism of **cholesterol** lowering caused by miR-122 inhibition in vivo, a cDNA microarray experiment was performed.

Table 1. Changes in gene expression after miR-122 ASO treatment of normal mice

	Microarray		RT-PCR	
Gene	Fold Change from Saline	p Value	Fold Change from Saline	p Value
Fatty-Acid Metabolism				
ACACA/ACC1	0.7	0.136	0.7	0.050
ACACB/ACC2	0.4	0.016	0.4	0.002
ACLY	0.3	0.005	0.4	0.002
DGAT1	1.1	0.462	1.1	0.085
DGAT2	0.9	0.384	1.1	0.196
FASN	0.6	0.153	0.6	0.092
LIPC	0.7	0.023	0.8	0.117
PPARGC1A	1.0	0.782	1.1	0.619
PPARA	1.3	0.172	0.9	0.572
SCD1	0.5	0.004	0.2	0.001
SREBP1	0.4	0.004	0.5	0.002
SREBP2	1.0	0.780	0.9	0.016
Cholesterol Metabolism				
CD36	1.4	0.247	0.7	0.014
HMGCR	0.8	0.129	0.8	0.154
LDLR	1.0	0.890	0.9	0.222
PMVK	0.4	0.007	ND	

Liver RNA from mice treated twice weekly for 4 weeks with 50 mg/kg miR-122 ASO or saline was used for a cDNA microarray experiment using GE CodeLink Mouse Whole Genome chips or for TaqMan RT-PCR confirmation of array results. n = 5. Shown are key genes related to lipid metabolism.

结果与分析

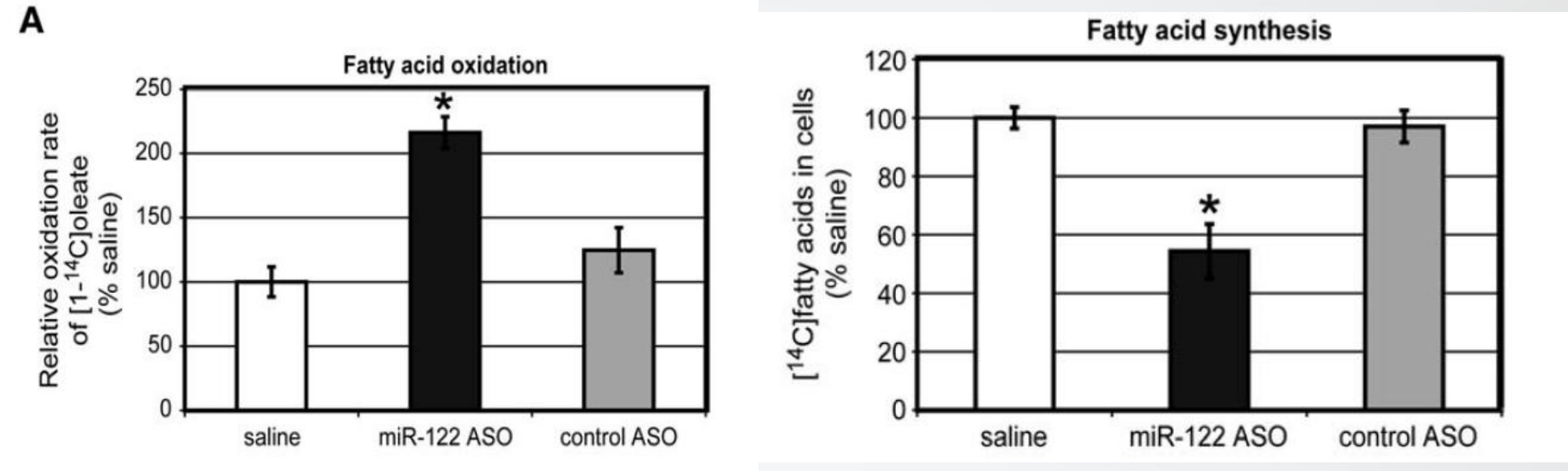
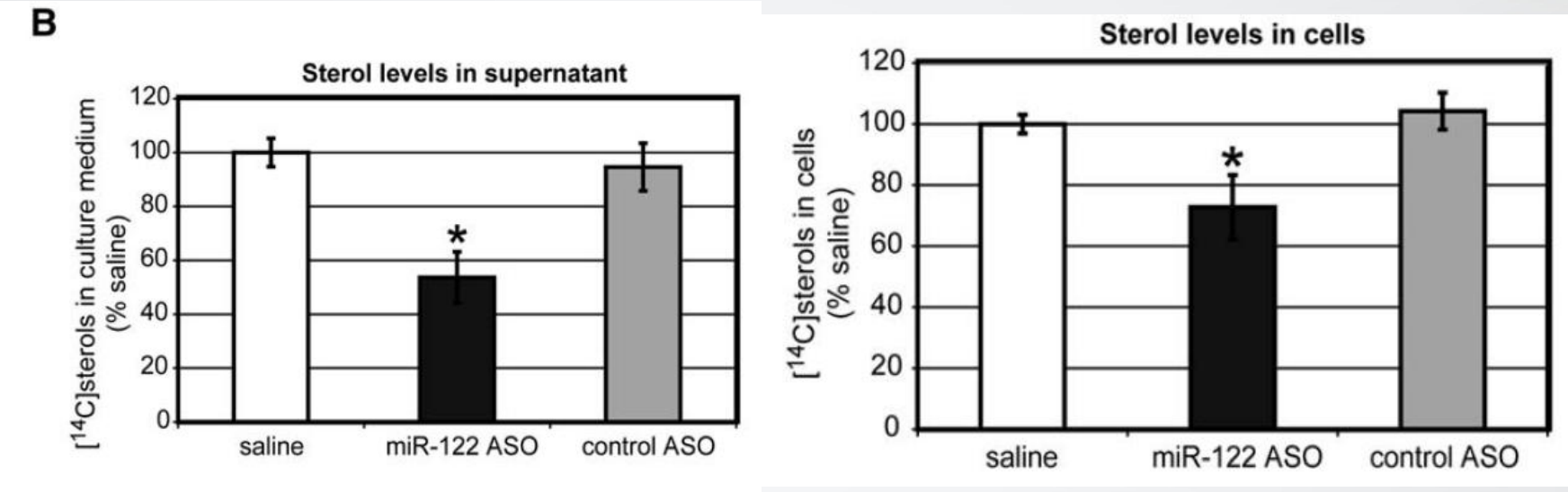


Figure 4. Ex vivo analysis of lipid metabolism after miR-122 inhibition
Hepatocytes isolated from mice treated with miR-122 ASO or a control ASO (25 mg/kg) twice weekly for 3 weeks were used for subsequent in vitro measurement of fattyacid and sterol synthesis rates and fatty-acid oxidation rates. Shown are results from five mice per group, with each measurement done in triplicate. Error bars = SEM.*$p < 0.05$.
A) Fatty-acid oxidation rate in isolated hepatocytes as measured by oxidation of [1-14C]oleate into 14CO2 and [14C]ASPs; de novo fatty-acid synthesis rate as measured by the amount of [14C]acetate incorporated into fatty acids.

结果与分析



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B) Sterol synthesis rate in isolated hepatocytes as measured by amount of [14C]acetate incorporated into sterols inside cells or secreted into the culture medium.

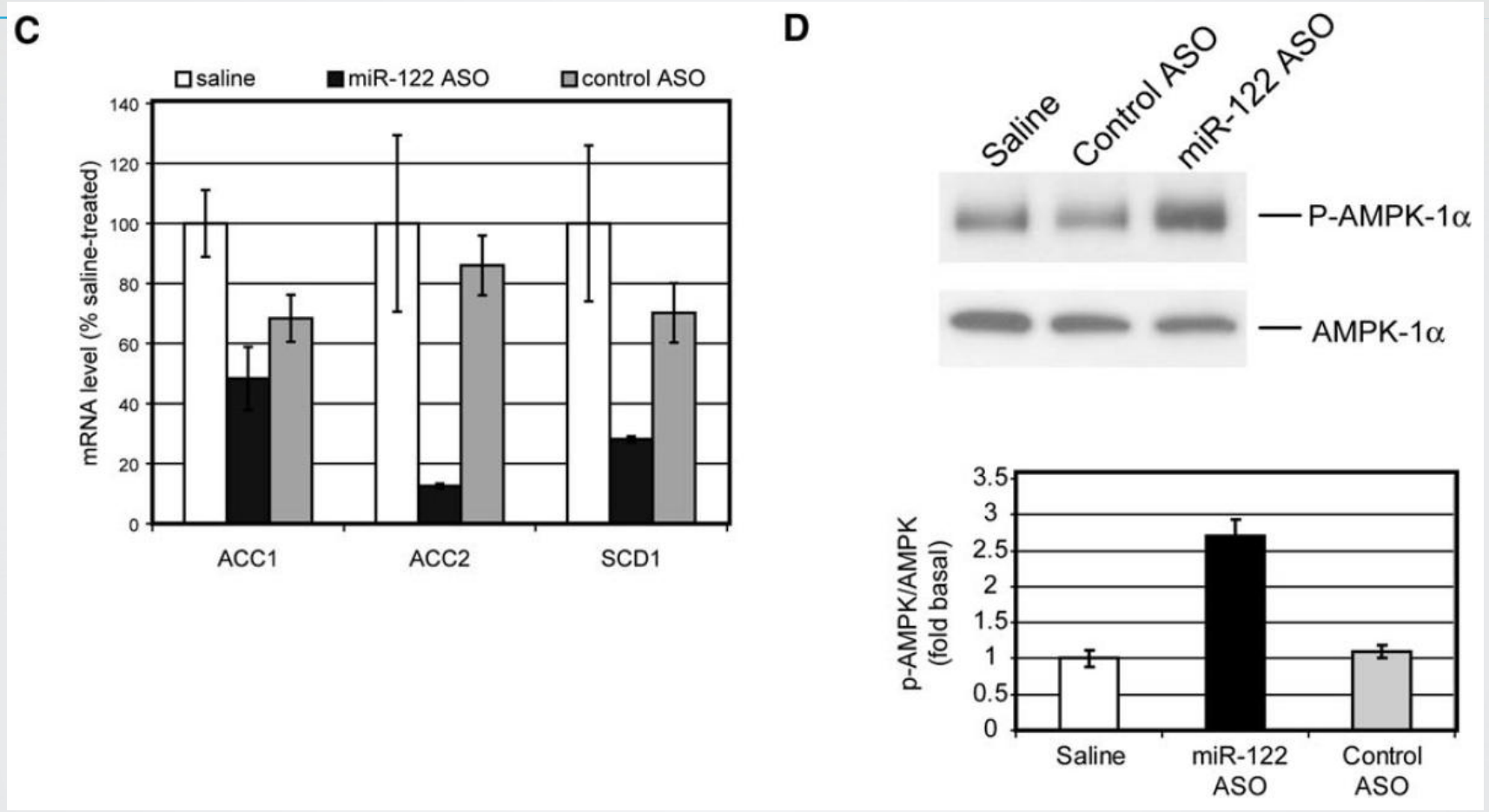


Figure 4. Ex vivo analysis of lipid metabolism after miR-122 inhibition
C) TaqMan RT-PCR measuring mRNA levels in isolated hepatocytes of genes involved in lipid metabolism. D) Western blotting for phospho-AMPKa1 catalytic subunit in liver extracts frommice treated with miR-122 ASO or control ASO in vivo twice weekly (50 mg/kg) for 4 weeks.The pictured gel shows pooled samples for each group, but quantitation is from n = 5 samples per group.

结果与分析

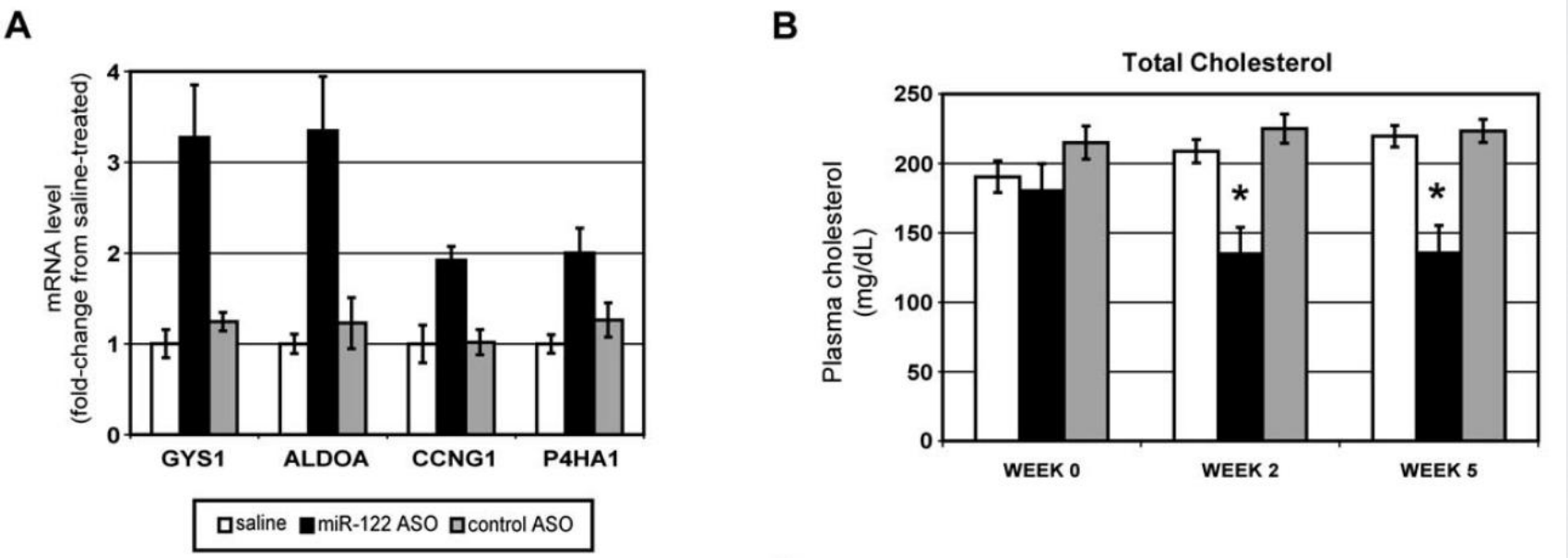


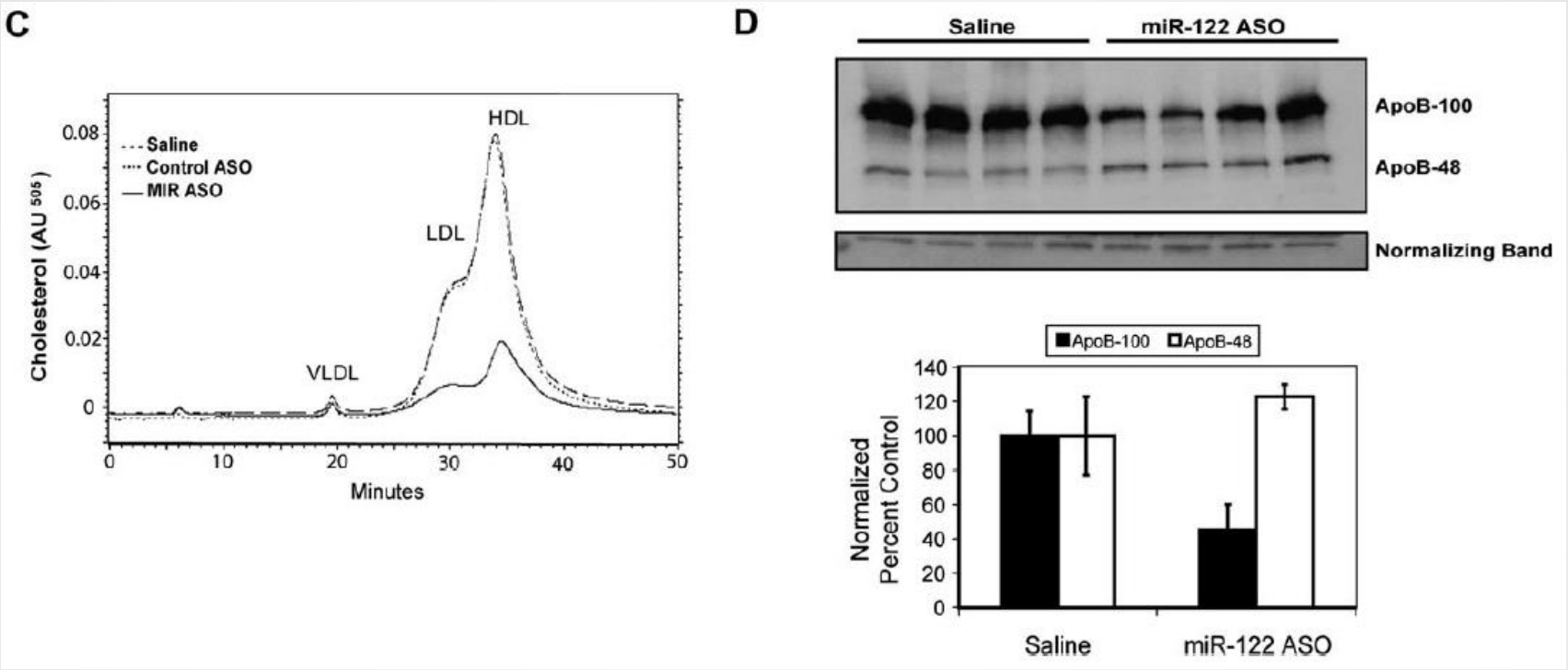
Figure 5. Cholesterol lowering in diet-induced obesity mouse model after miR-122 inhibition

C57Bl/6 mice that had been fed a high-fat diet for 19 weeks were treated s.c. with 12.5 mg/kg miR-122 or control ASO twice weekly for 5 1/2 weeks. n = 5.

A) TaqMan RT-PCR measuring levels of miR-122 target genes in liver RNA after treatement. Error bars = SD.

B) Plasma cholesterol levels at various time points after start of treatment. Error bars = SEM. *$p < 0.05$.

结果与分析



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C57Bl/6 mice that had been fed a high-fat diet for 19 weeks were treated s.c. with 12.5 mg/kg miR-122 or control ASO twice weekly for 5 1/2 weeks. n = 5.

C) High-performance liquid chromatography analysis of lipoprotein profiles in treated mice.

D) Plasma ApoB-100 and ApoB-48 levels measured by Western blotting and normalized to nonspecific band. Graph represents the average of four samples per group with the standard deviation indicated.

结果与分析

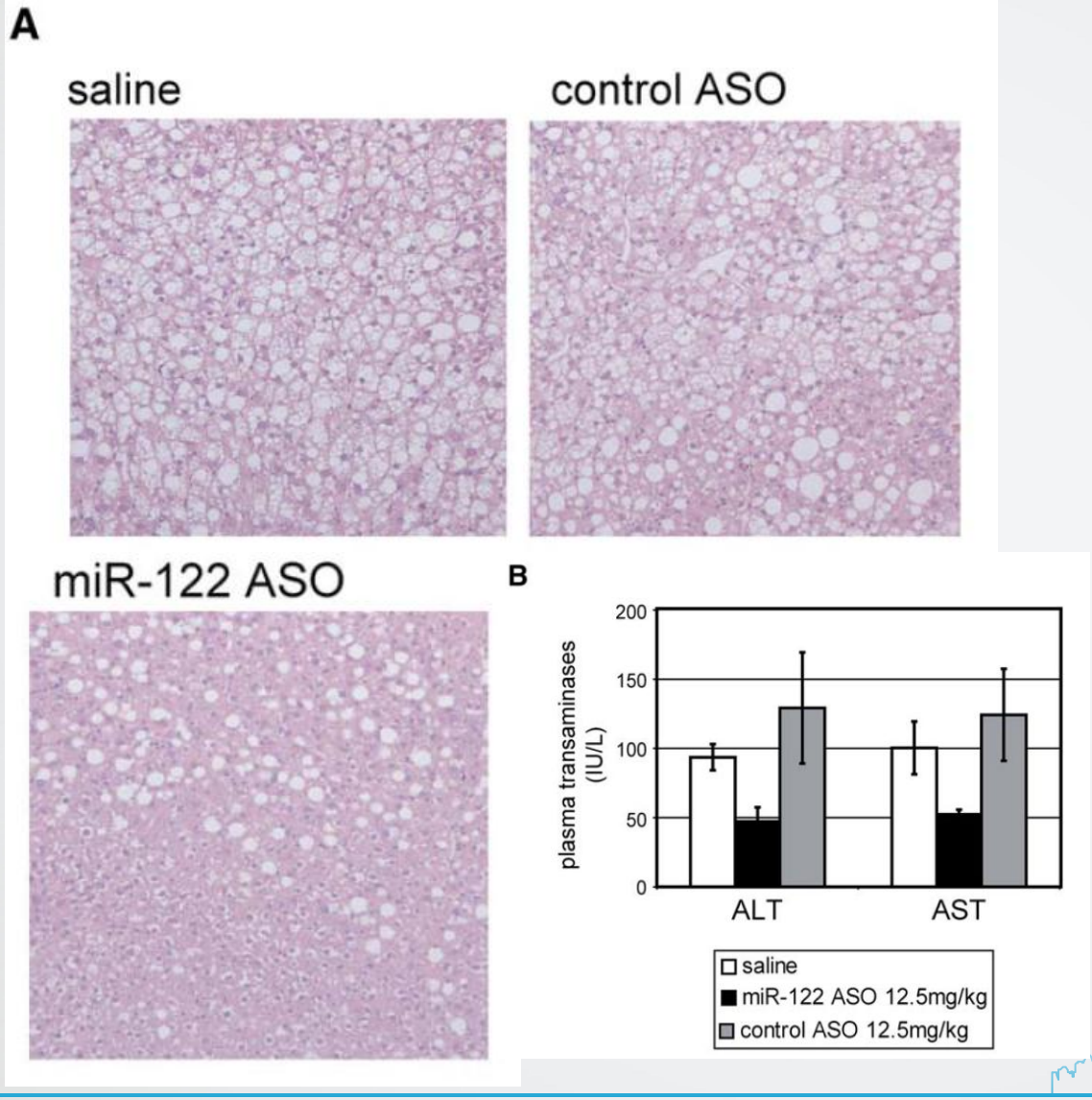
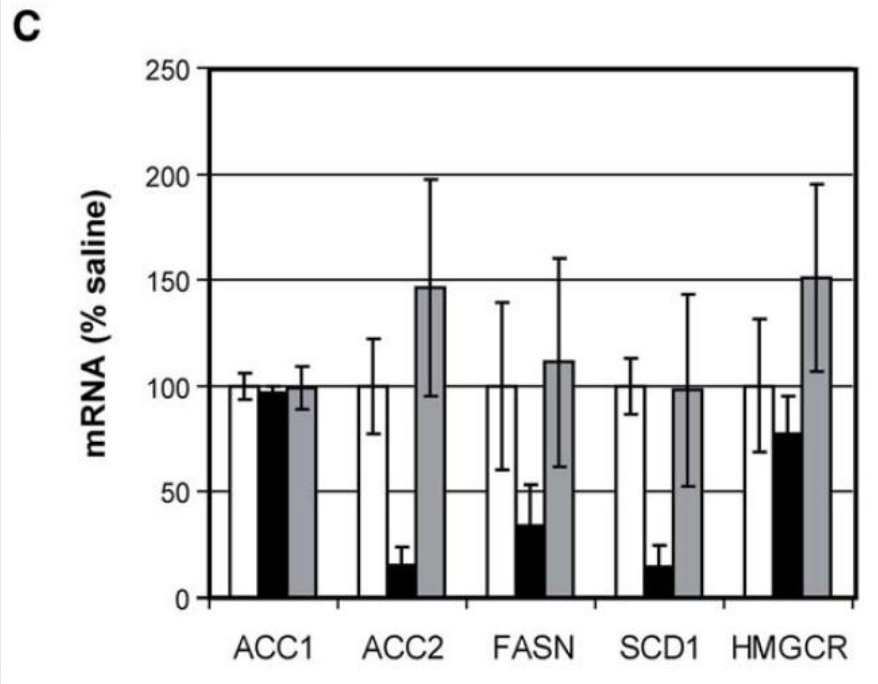
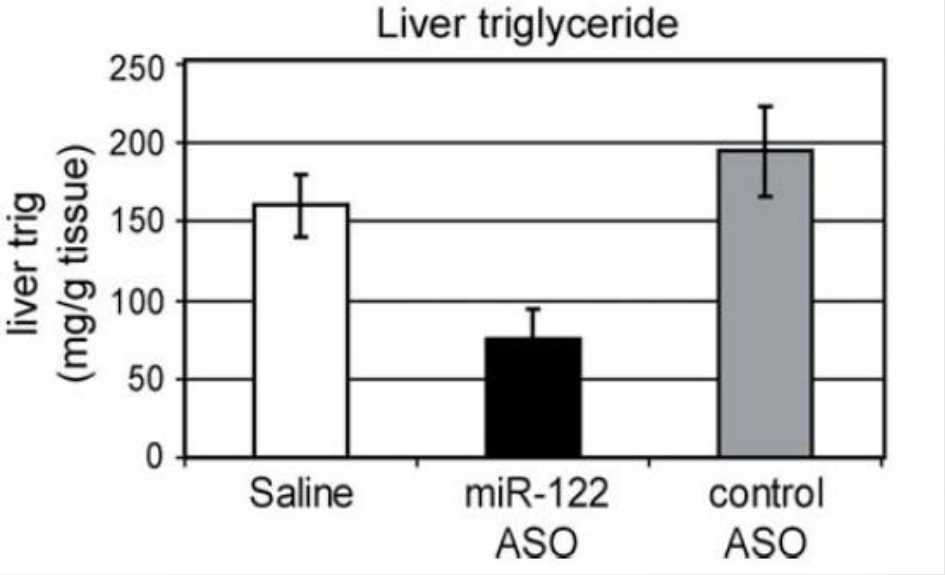


Figure 6. Improvement in liver steatosis in high-fat-fed mice after miR-122 inhibition
C57Bl/6 mice that had been fed a high-fat diet for 19 weeks were treated s.c. with 12.5 mg/kg miR-122 or control ASO twice weekly for 5 1/2 weeks. n = 5. Error bars = SEM.
A) Representative hematoxylin-and-eosin-stained liver sections photographed at 203 magnification and hepatic triglyceride content after 5 1/2 weeks of ASO treatment.
B) Plasma transaminase levels measured after 5 1/2 weeks of ASO treatment.

结果与分析





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C) TaqMan RT-PCR evaluating liver mRNA levels of genes involved in lipid metabolism.

（四）反思与体会

1.多浓度设置来验证miR-122靶基因

2.两种活体模型

normal mice　　diet-induced obesity mouse model

3.两种细胞类型

primary mouse hepatocytes　　mouse liver carcinoma

AML12 cell line

4.组织切片染色

Representative hematoxylin-and-eosin-stained liver sections

（四）反思与体会

5.不同的实验周期及注射剂量

twice weekly for 4 weeks　　twice weekly for 3 weeks

twice weekly for 5 1/2 weeks

6.对miR-122的检测

Northern blotting for miR-122 in liver RNA

7.靶基因对miRNA敏感性研究

P4HA1 mRNA appeared most sensitive to miR-122 inhibition, as it displayed maximal upregulation at the lowest ASO dose tested.

谢谢大家！