读书报告

张文雅

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MicroRNA-122 Inhibits Lipid Droplet Formation and Hepatic Triglyceride Accumulation via Yin Yang 1

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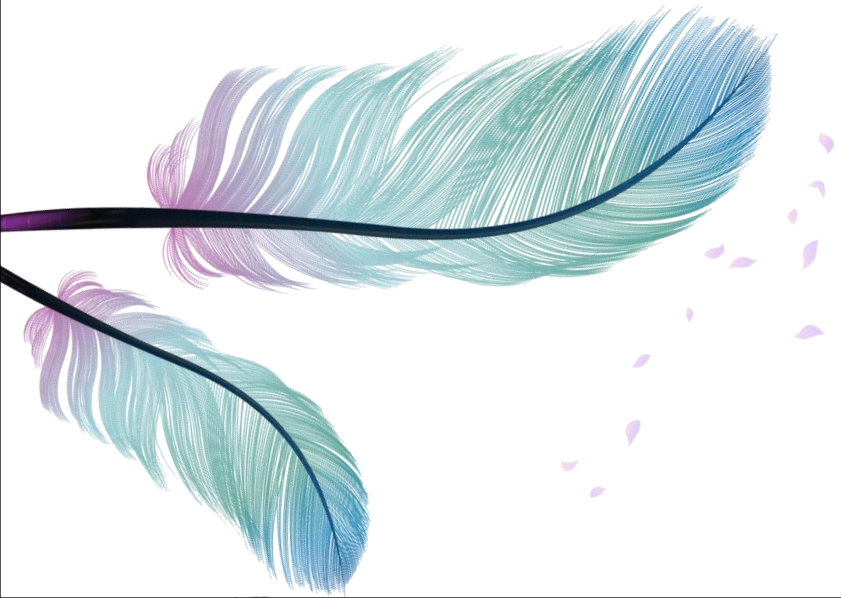
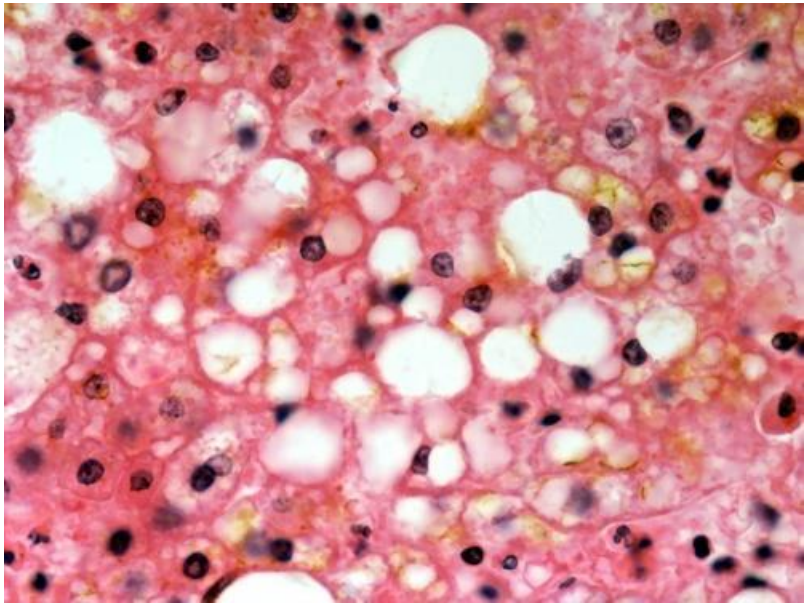


01

背景及意义

(Background &Significance)



非酒精性脂肪性肝病（NAFLD）是以肝细胞脂肪变性和脂质蓄积为主要特征的临床病理综合征。是一种与胰岛素抵抗（IR）和遗传易感性密切相关的代谢应激性肝损伤，疾病谱包括单纯性脂肪肝（NAFL）、脂肪性肝炎（NASH）及其相关肝硬化和肝细胞癌。

肝细胞中TG含量过多是由于游离脂肪酸（FFA）进入肝脏的增加，从而导致NAFLD的发展。在肥胖相关的NAFLD中，由于脂肪组织胰岛素抵抗，FFA对肝脏的传递增加，特别是在喂食状态下。肝细胞损伤是由FFAs及其衍生物的脂质毒性以及线粒体能力超载所致。

肝脂变受不同途径的调节，包括固醇调节元件结合蛋白1(SREBP-1)，肝X受体(LXR)，成纤维细胞生长因子21(FGF 21)，以及碳水化合物反应元件结合蛋白(ChREBP)。SREBP-1c是由一个核受体级联调控的，这一过程涉及法尼类X受体(FXR)和小异源二聚体(SHP)。研究表明，YY1通过靶向FXR基因的内含子1来调节脂质代谢的动态平衡。此外，FXR还参与调节胰岛素抵抗和脂质代谢紊乱。FXR能抑制肝星状细胞的活化、炎症细胞的侵袭，以及肝细胞的再生，从而防止肝纤维化的发生。目前已经确定了几种FXR激动剂，表明YY1-FXR在防治NAFLD方面有一定的应用前景。

YY1是多梳组蛋白家族中的一员，它是一种转录因子，在发育、复制和细胞增殖等生物学过程中起着重要的作用。

02

材料与方法
(Materials and Methods)

① An FFA fat-overloading model

实验组：oleic acid : palmitate acid = 2:1

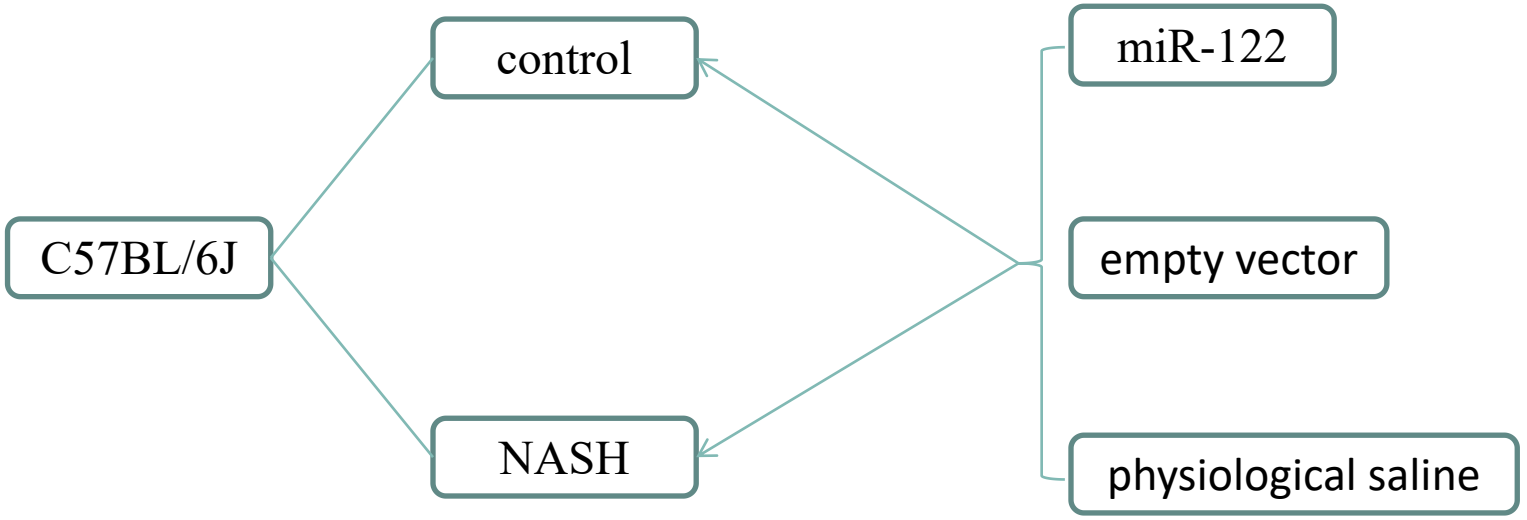
对照组：treated with 1% fatty acid-free BSA without palmitate acid and oleic acid.

② Analysis of lipid content

cells were fixed in 10% formalin at 4°C for 10 min and the slides were rinsed with PBS (pH 7.4). After air drying, the slides were placed in 100% propylene glycol for 2 min and stained with a 0.5% Oil Red O solution in propylene glycol for 30 min. The slides were transferred to an 85% propylene glycol solution for 1 min, rinsed in distilled water twice, and processed for hematoxylin counterstaining.

③ Cell viability MTT assay

20 μL MTT solution were added to each well and the plate was subsequently incubated at 37°C for 4 h in the incubator.The liquid was then removed from the plate and 150 μL DMSO were added to each well. All plates were read at 490 nm.



STZ(链脲佐菌素) 4 weeks
feed HFD

every 3 days for a
total of five times

YY1-WT-F

CTCATCTCTTCTGTCCTTTCCTGTCTCTGAAATAGTCATCACTCCCCTTGACTCTCTCTGTTCACGTCTC

YY1-WT-R

TCGAGAGACGTGAACAGAGAGAGTCAAGGGGAGTGATGACTATTTCAGAGACAGGAAAGGACAGAA

GAGATGAGAGCT

YY1-MUT-F CTCATCTCTTCTGTCCTTTCCTGTCTCTGAATATGTGTAGTGAGGCCTTGACTCTCTCT

GTTCACGTCTC

YY1-MUT-R TCGAGAGACGTGAACAGAGAGAGTCAAGGCCTCACTACACATATTCAGAGACAGGAAA

GGACAGAAGAGATGAGAGCT

YY1-PC-F CCAAACACCATTGTCACACTCCAACCGGTCAAACACCATTGTCACACTCCAC

YY1-PC-R TCGAGTGGAGTGTGACAATGGTGTTTGACCGGTTGGAGTGTGACAATGGTGTTTGGAG

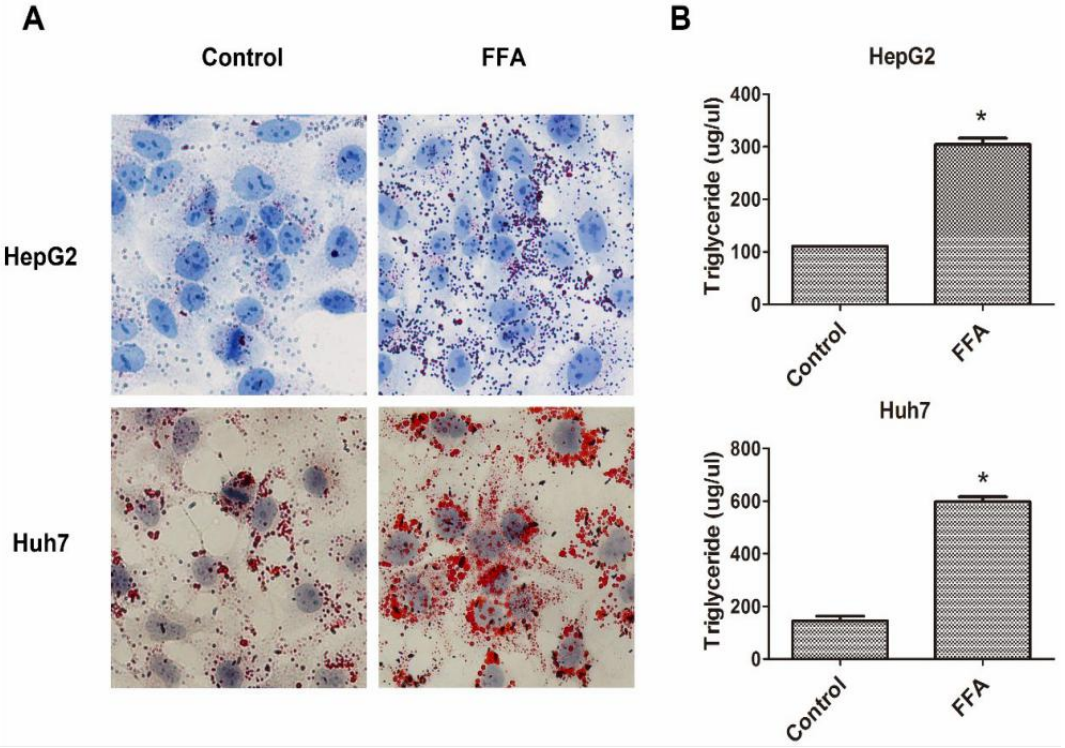
03

研究结果
(Results)

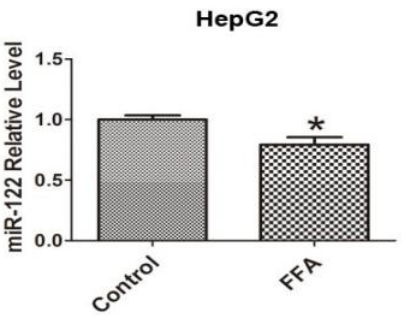
Effect of FFA on lipid accumulation in HepG2 and Huh7 cells

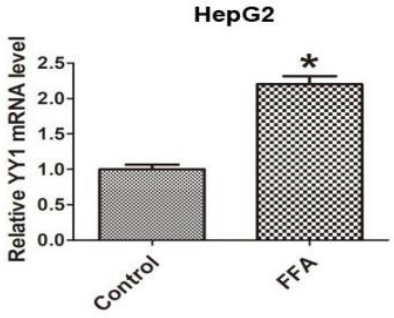
0.5mM FFA

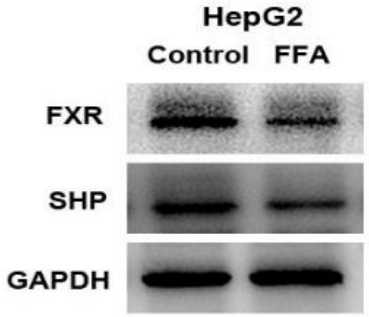
24h

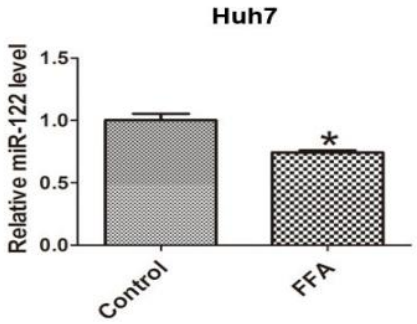


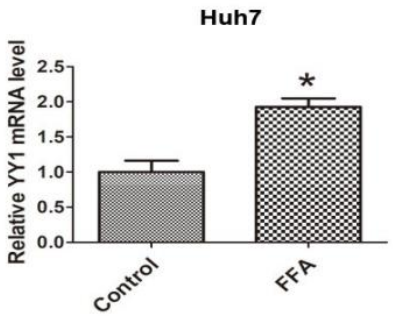
Differential expression of miR-122 and FXR in steatotic hepatocytes

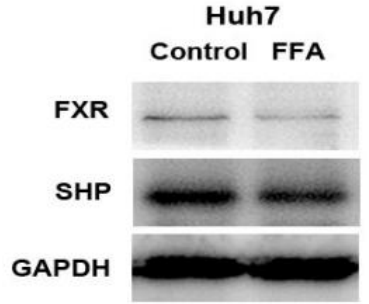




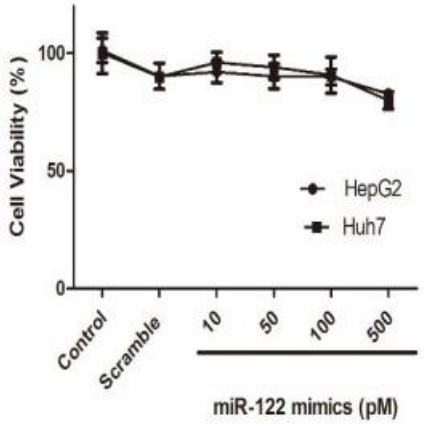


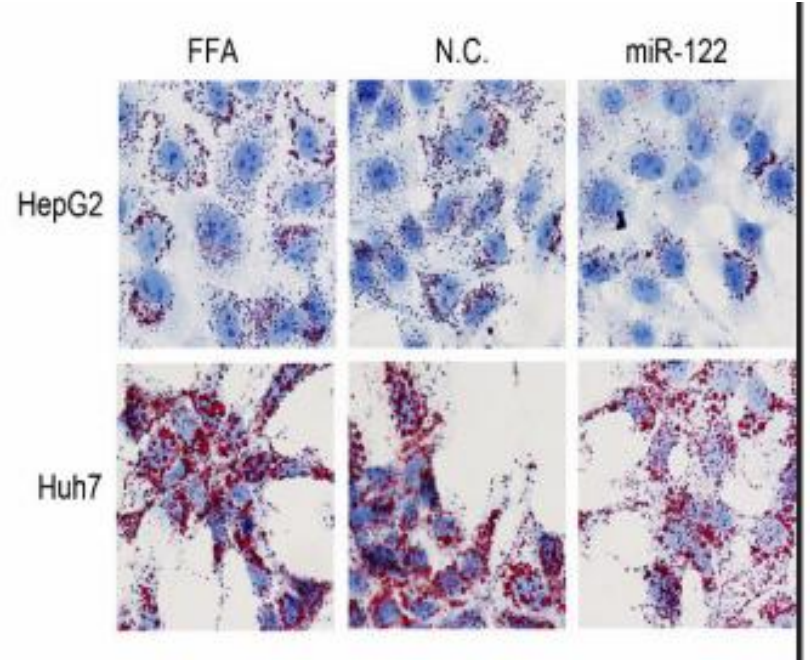


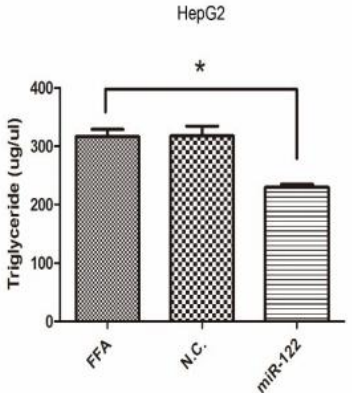


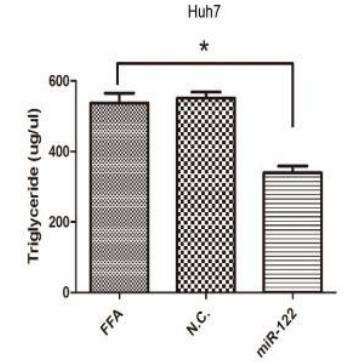


miR-122 regulates lipid droplet formation and TG content in HepG2 and Huh7 cells

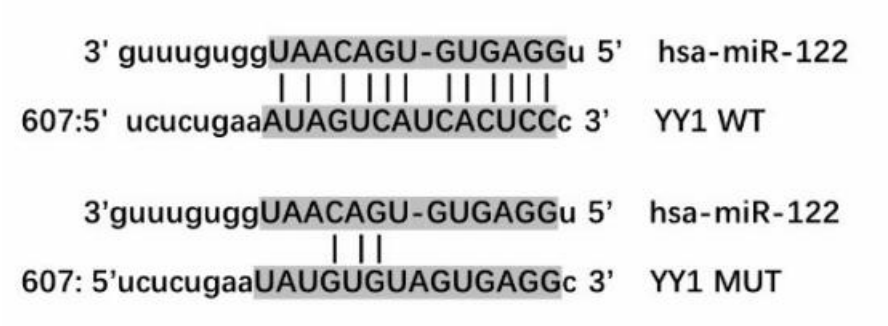


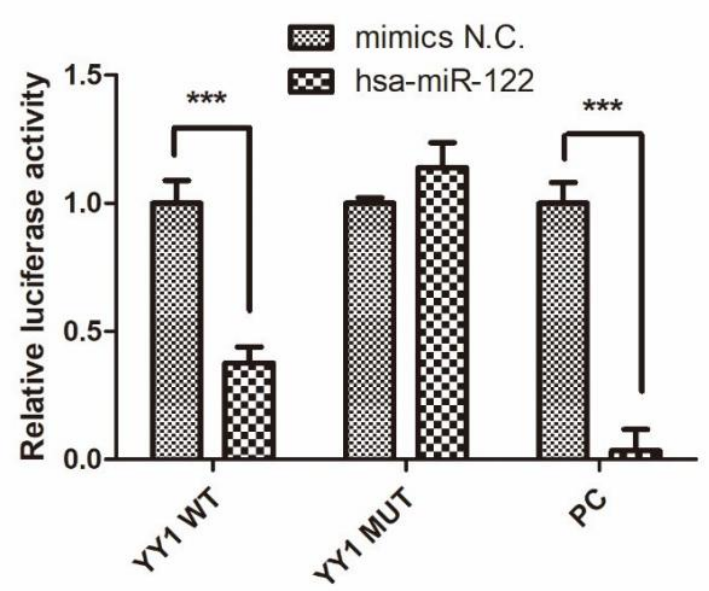




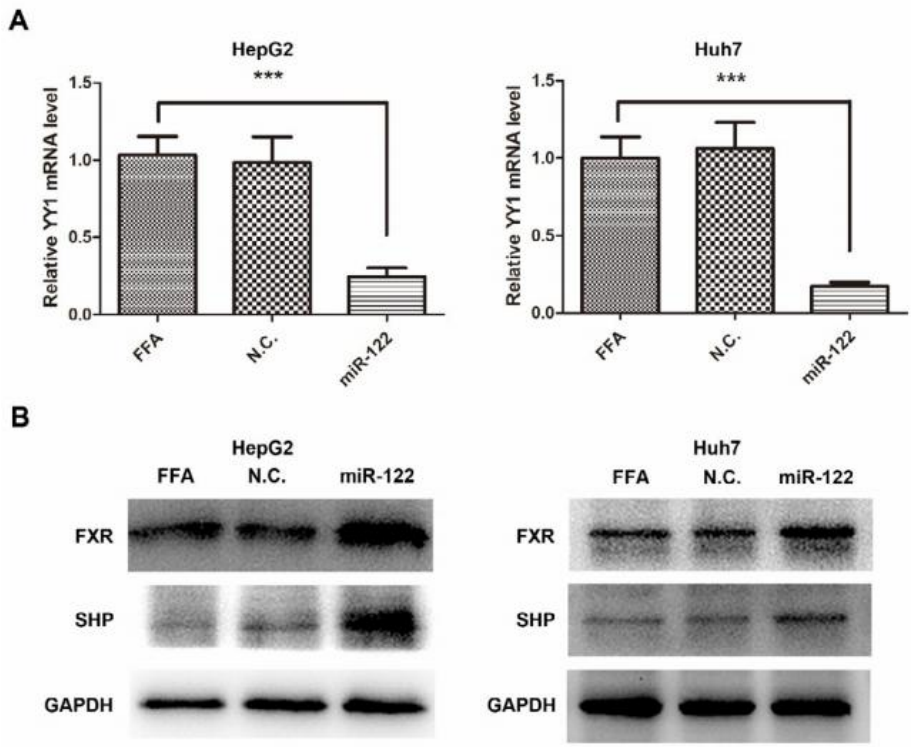


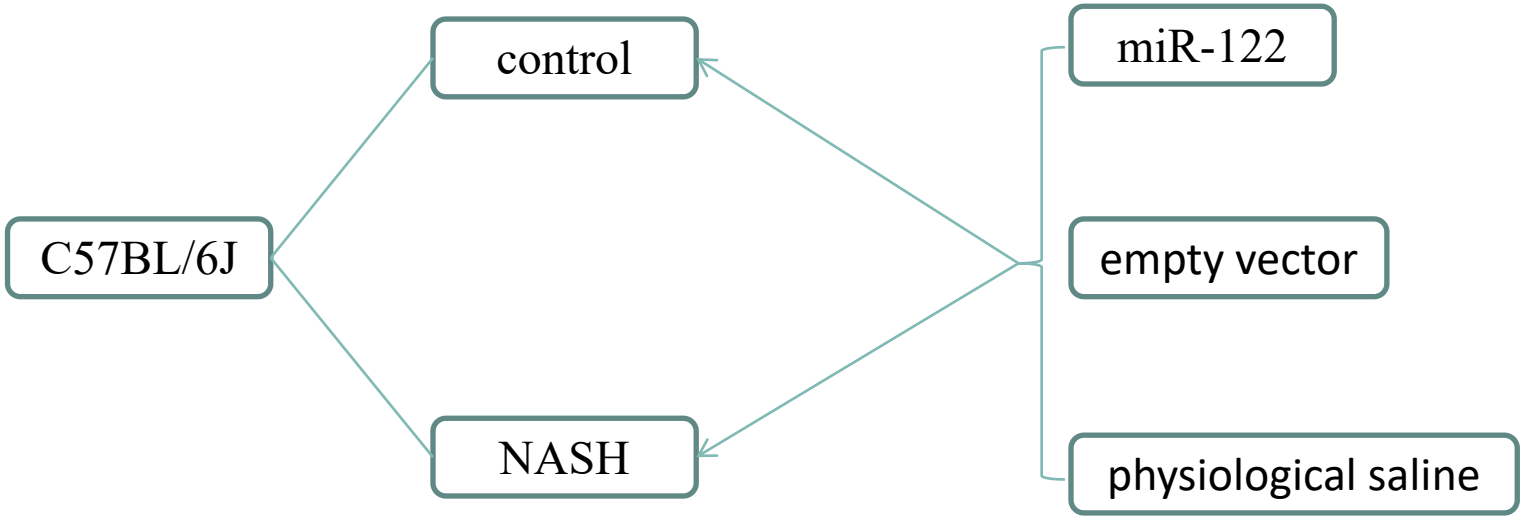
Confirmation of the YY1 3'UTR as a target of miR-122 by luciferase reporter assays





miR-122 mimics regulate YY1 and FXR levels in HepG2 and Huh7 cells





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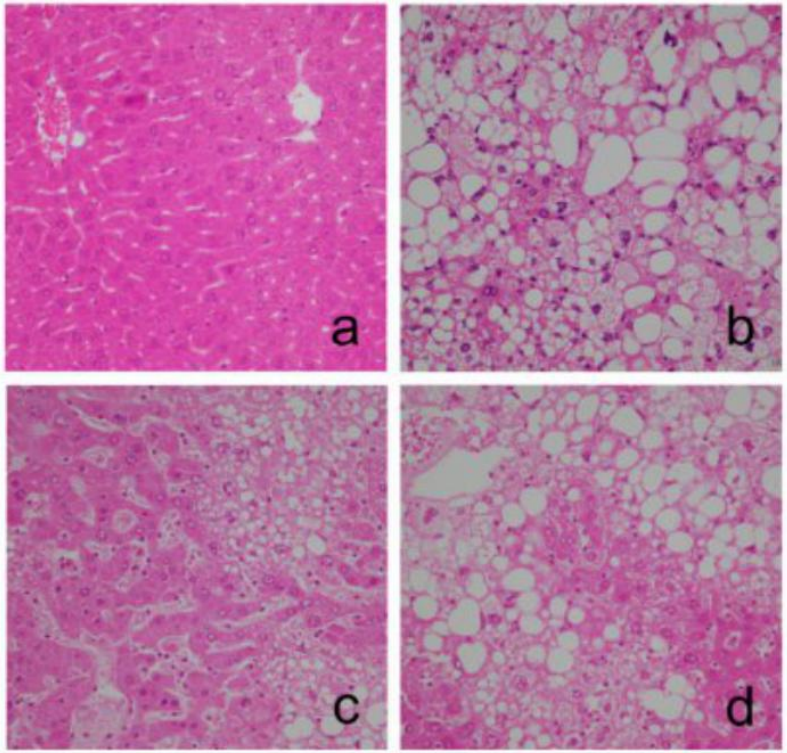
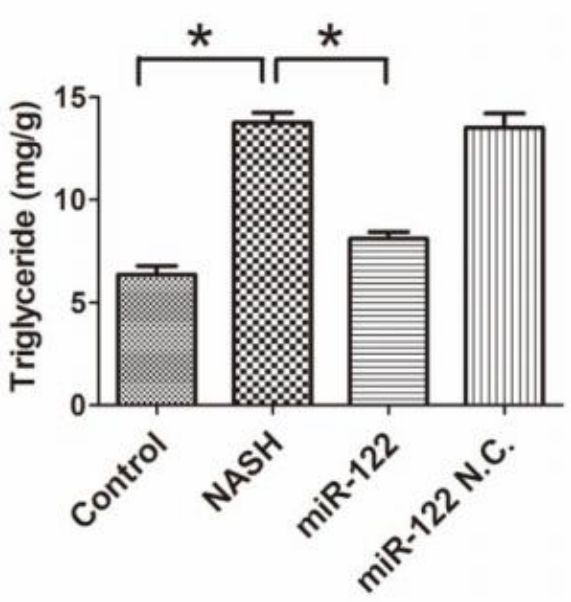
miR-122 inhibits hepatic TG accumulation and improves the histology of NASH mice





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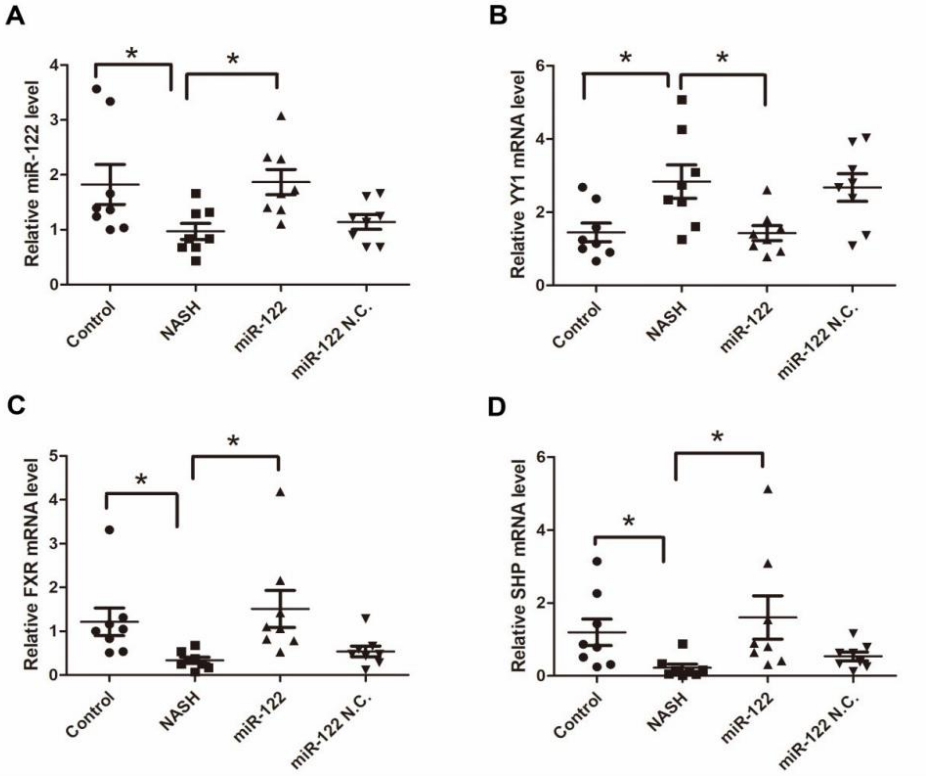

04

总结分析
(Discussion)

FXR是核受体超家族的成员，FXR在FXR缺乏小鼠TG代谢中的作用得到了证实，其表现为明显的肝骨病和高血糖。合成的FXR激动剂能预防肥胖小鼠肝脏脂肪变性。然而FXR mRNA的3′UTR中没有miR-122结合序列。推测mir-122可能间接导致FXR通过抑制其抑制剂的表达，提出YY1可能是miR-122在肝脏中的靶点。研究mir-122对肝细胞脂质沉积和TG稳态的调节作用对了解NAFLD发病机制的重要意义。

敬请各位批评指正！