



读书报告

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职韶阳

2018年12月9日



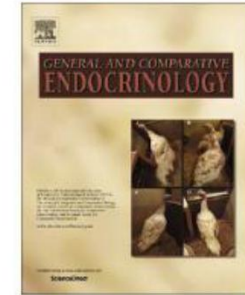


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Research paper

Small interfering RNA mediated knockdown of irisin suppresses food intake and modulates appetite regulatory peptides in zebrafish

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CrossMark

鸢尾素的小干扰RNA介导抑制斑马鱼的食物摄取以及影响食欲调节肽

IF=2.564

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研究背景





运动可产生一种分泌性蛋白质分子Fndc5，能提高具有燃烧能量功能的浅棕色脂肪数量

Fndc5基因的mRNA序列在人与小鼠之间完全相同，它与胰岛素之间有85%的同源性，而与胰高血糖素之间有90%同源性

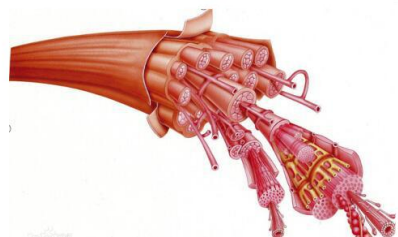
肥胖小鼠接受带有Fndc5基因的腺病毒转染之后，增加了葡萄糖耐量和能量的动态平衡

Fndc5在肌肉、心脏和中枢神经系统中很丰富

Fndc5是运动和PGC-1 α 诱导的蛋白质，被切割释放出多肽-Irisin

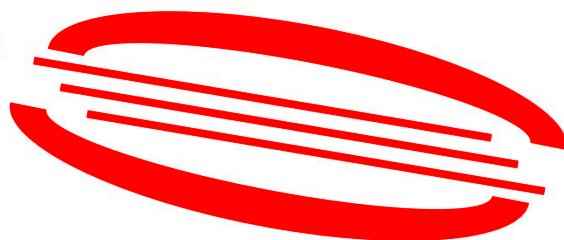


Irisin

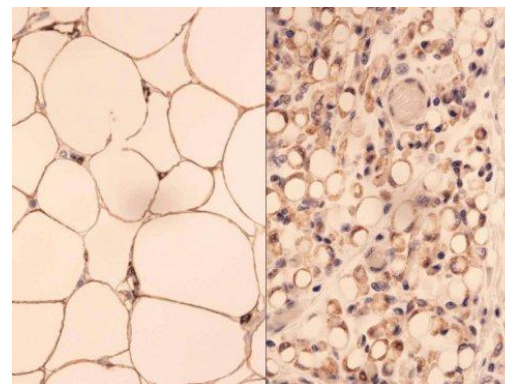


骨骼肌

血液循环



白色脂肪



脂肪组织
FND5mRN
A被激发,, 合
成Irisin。



棕色脂肪



斑马鱼以及食欲调节肽



斑马鱼(Barchydanio rerio)

1、俗称“花条鱼”、“蓝条鱼”。鱼纲，鲤科。原产印度和孟加拉。是一种性情活泼、不怕冷的热带鱼品种。

2、斑马鱼基因与人类基因的相似度达到87%，意味着其实验结果大多数情况下适用于人体，作为模式生物的优势很突出。

3、雌性斑马鱼可产卵数百枚，胚胎在24小时内就可发育成形。

4、斑马鱼具有养殖方便、繁殖周期短、产卵量大、胚胎体外受精、体外发育、胚体透明的特点。

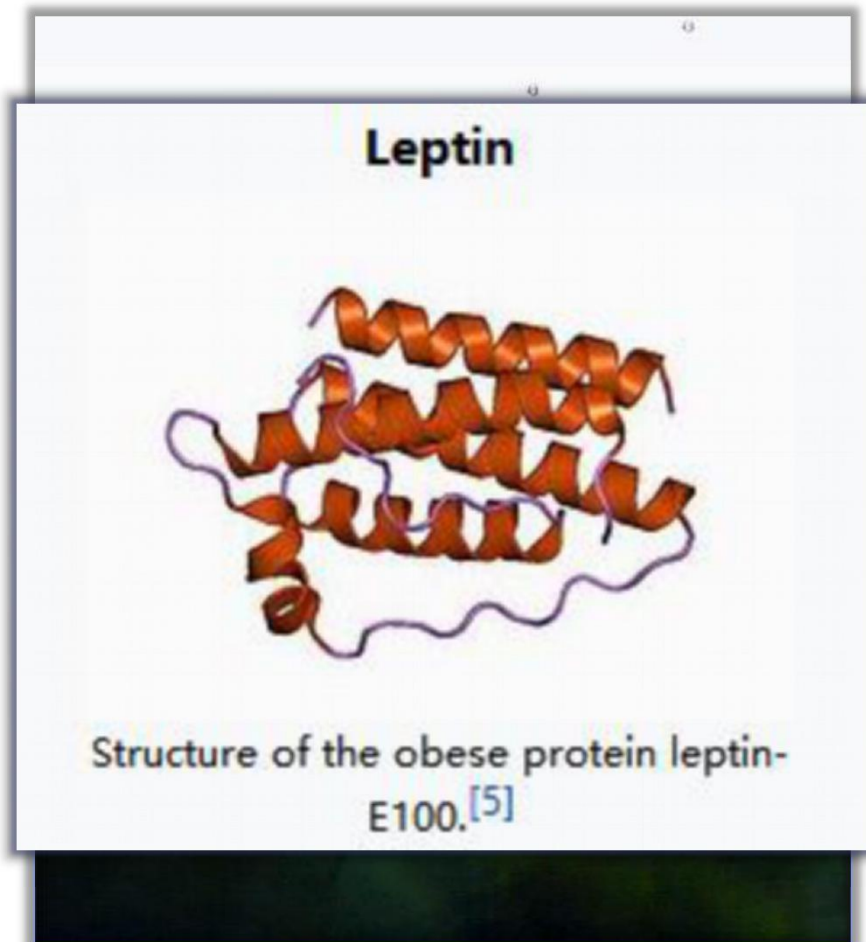
常见的食欲调节肽：

Orexin (食欲素)

NPY (神经肽Y)

CART

Ghrelin (胃饥饿素) leptin (瘦素)



研究目的



一、确定内源性鸢尾素和鸢尾素给药对食物摄入和食欲调节肽表达的可能作用

- (①描述斑马鱼中鸢尾素的组织特异性表达)
- (②鸢尾素给药或鸢尾素抑制是否对斑马鱼的食物摄入有任何影响)
- (③鸢尾素对斑马鱼中编码食欲调节肽的mRNA表达的影响)

二、首次显示内源性鸢尾素在调节斑马鱼食物摄入和食欲调节肽方面的作用

总而言之就是：①在斑马鱼中鸢尾素对摄食和食欲调节肽有没有作用。
②在斑马鱼中加入siRNA会干扰内源鸢尾素，其对摄食和食欲调节肽有没有作用。





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材料与amp;方法



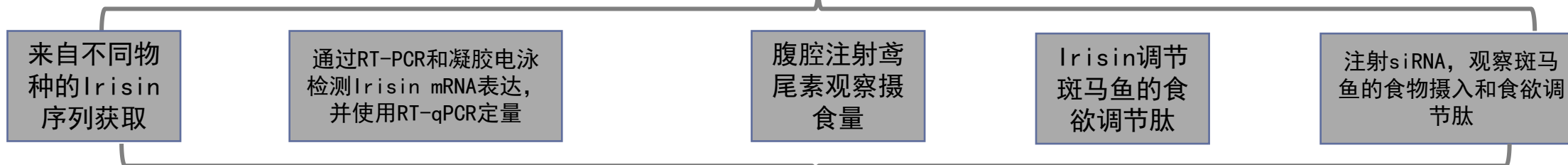
材料与方法



斑马鱼
标准长度：11±0.5cm，体重：50±5.0g

27°C，12小时黑暗/
12小时光照

所有鱼类每天上午十点喂食一次，体重3-4%，
饲喂商业颗粒饲料（缓沉颗粒）



Irisin和siRNA对斑马鱼的食物摄入及食
欲调节肽的调控作用

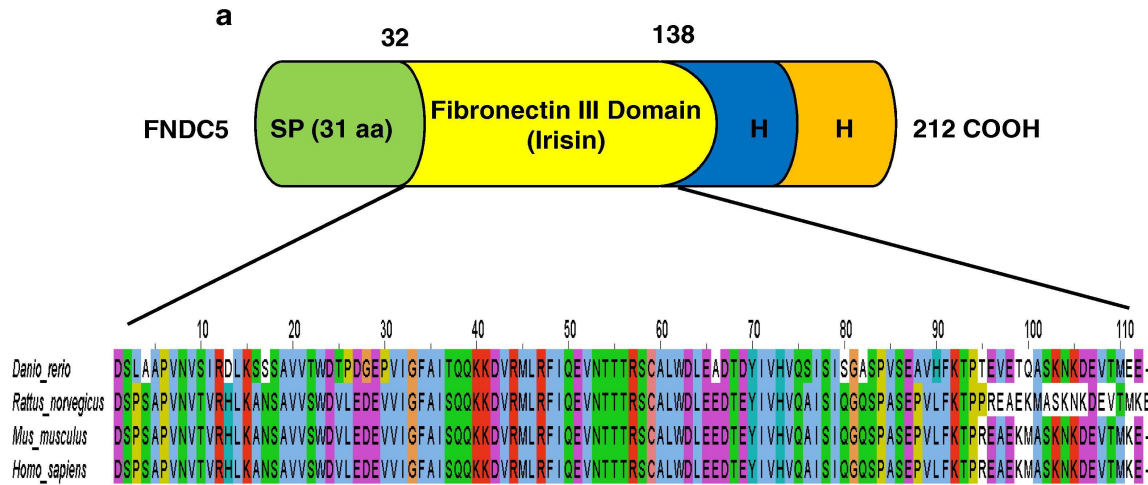


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结果与讨论



对FNDC5序列和鸢尾素的相对mRNA表达进行分析

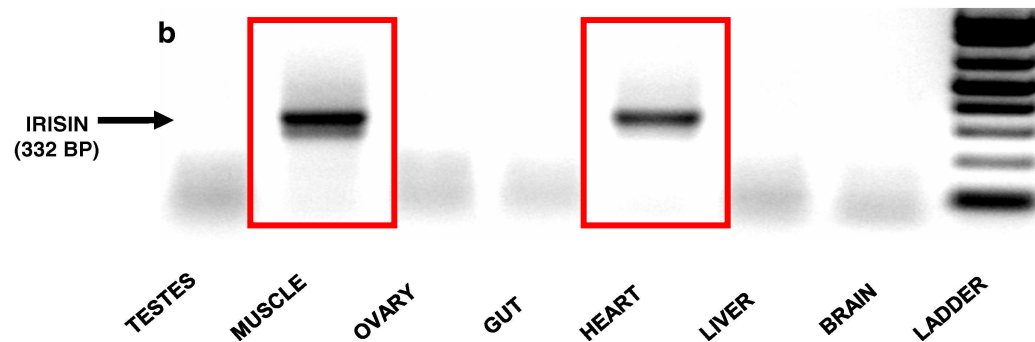


Sequence analysis found a very highly conserved irisin (32–138) in zebrafish.

Zebrafish irisin sequence exhibits 70–80% amino acid sequence identity to human, rat and mouse irisin sequences

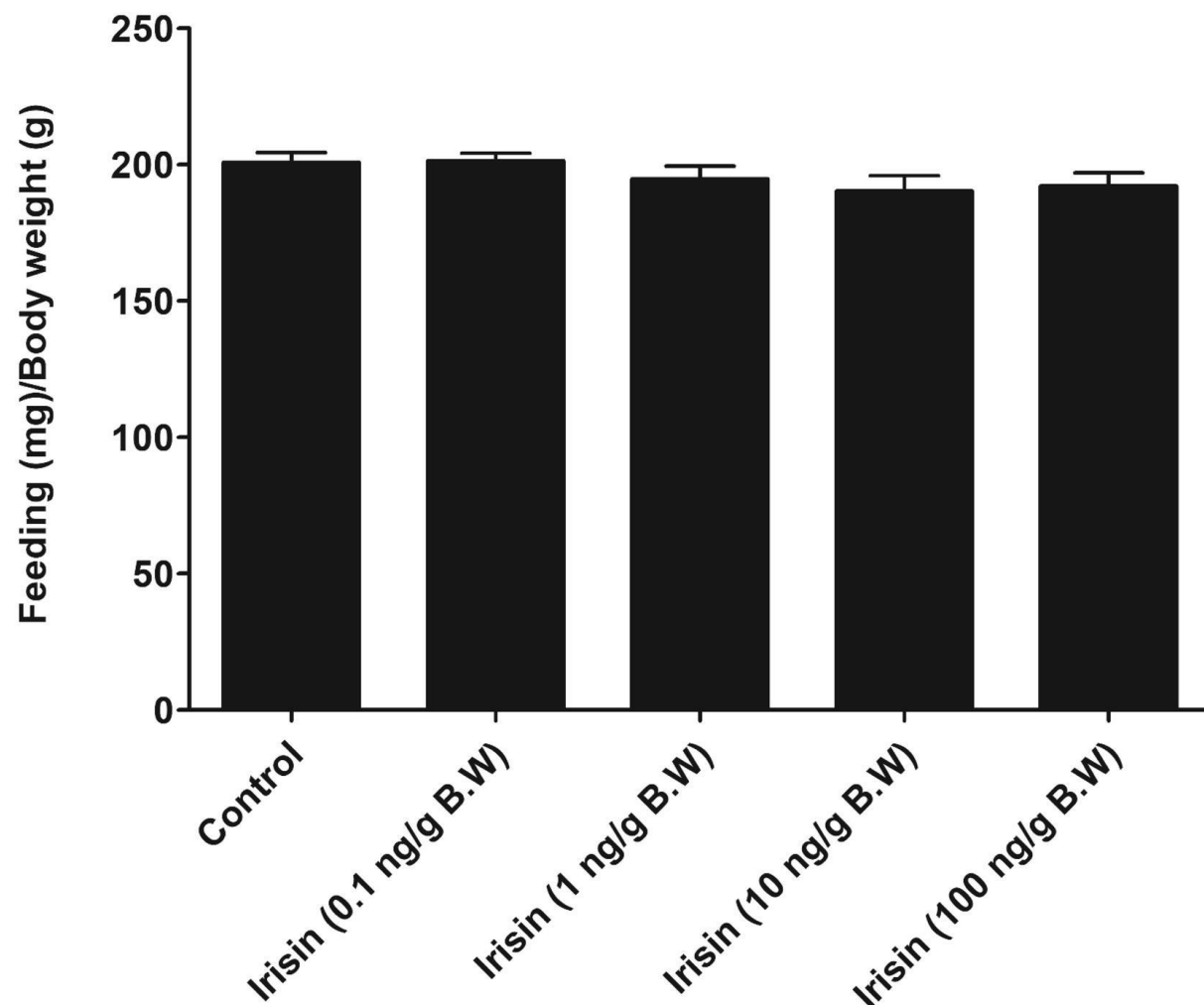
Abundance of irisin mRNA expression (332 bp) was detected in the skeletal and cardiac muscles of zebrafish

No amplicon was detected in other tissues (i.e. brain, liver, gut, ovary and testes) of zebrafish



Gel electrophoresis of irisin in zebrafish by reverse transcriptase PCR

腹腔注射Irisin不会影响斑马鱼的食物摄入量

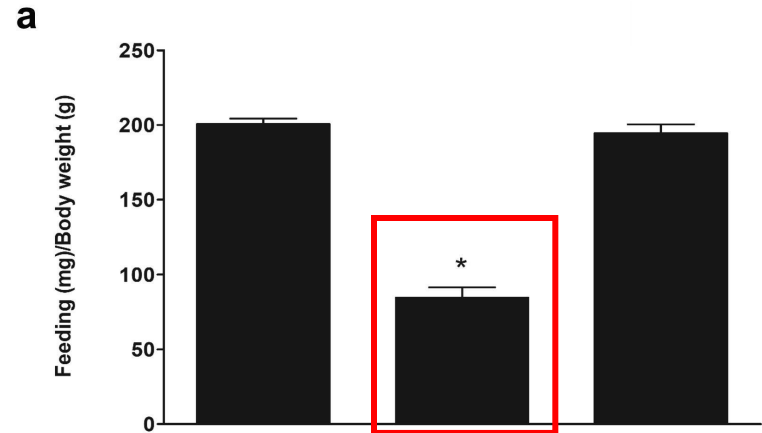


Synthetic human irisin was injected at 0.1, 1, 10 and 100 ng/g body weight (BW) just prior to their scheduled feeding time (10 AM).

Immediately after the administration of irisin or saline, fish were allowed to recover and were fed a pre-weighed quantity of food, and the food recovered 1 h post-administration, dry to quantify food intake

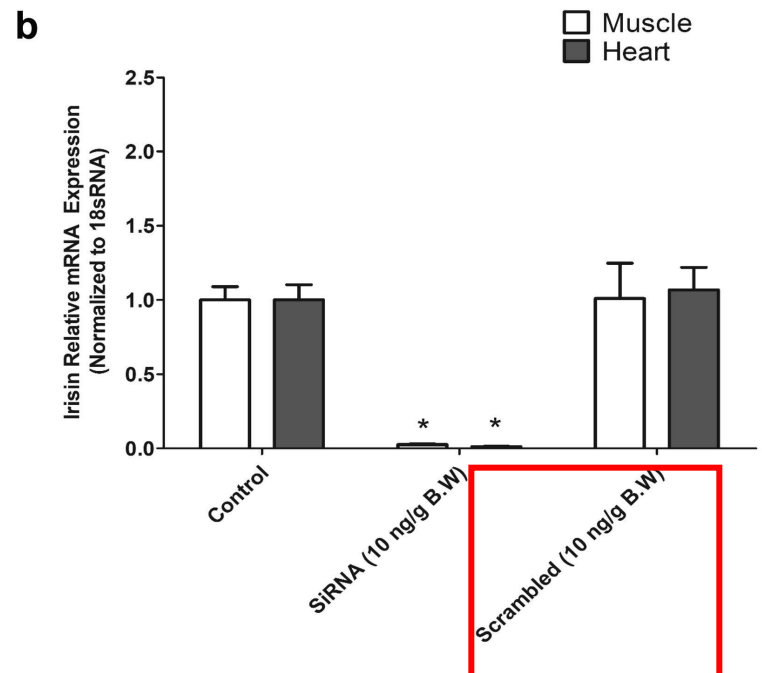
Intraperitoneal injection (i.p) of irisin had no effects on food intake in zebrafish.

siRNA对Irisin的抑制降低了斑马鱼的食物摄入量



Knockdown of irisin by siRNA (10 ng/g B.W) reduced food intake in zebrafish

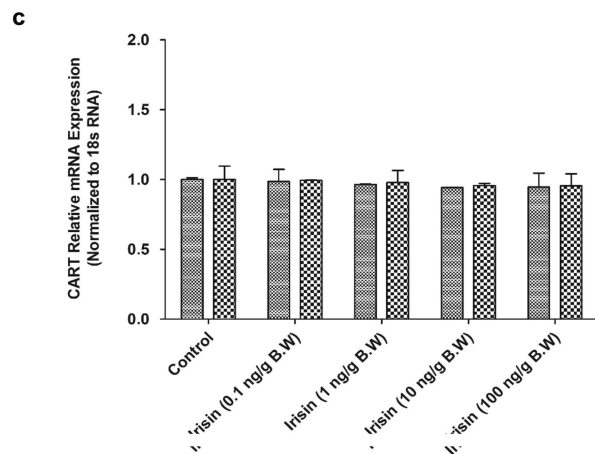
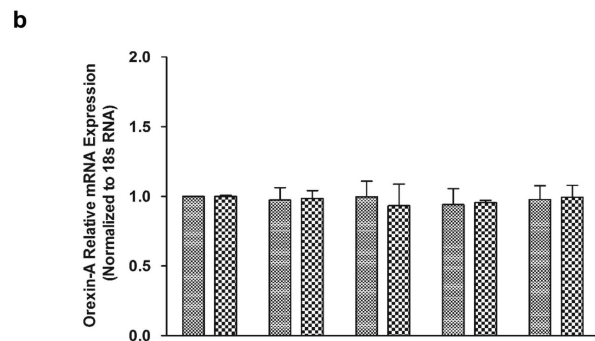
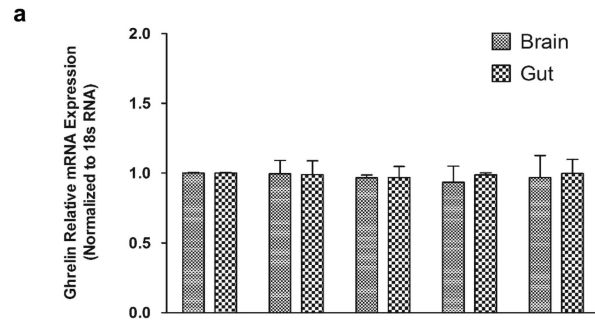
No effect on food intake was observed in response to an intraperitoneal injection of 10 ng/g B.W **scrambled** irisin siRNA



Irisin mRNA expression was reduced by **90%** in zebrafish skeletal muscle and heart of irisin siRNA treated fish, when compared to control (saline-injected) groups

No significant effect on irisin relative mRNA expression was observed in response to an intraperitoneal injection of scrambled irisin siRNA

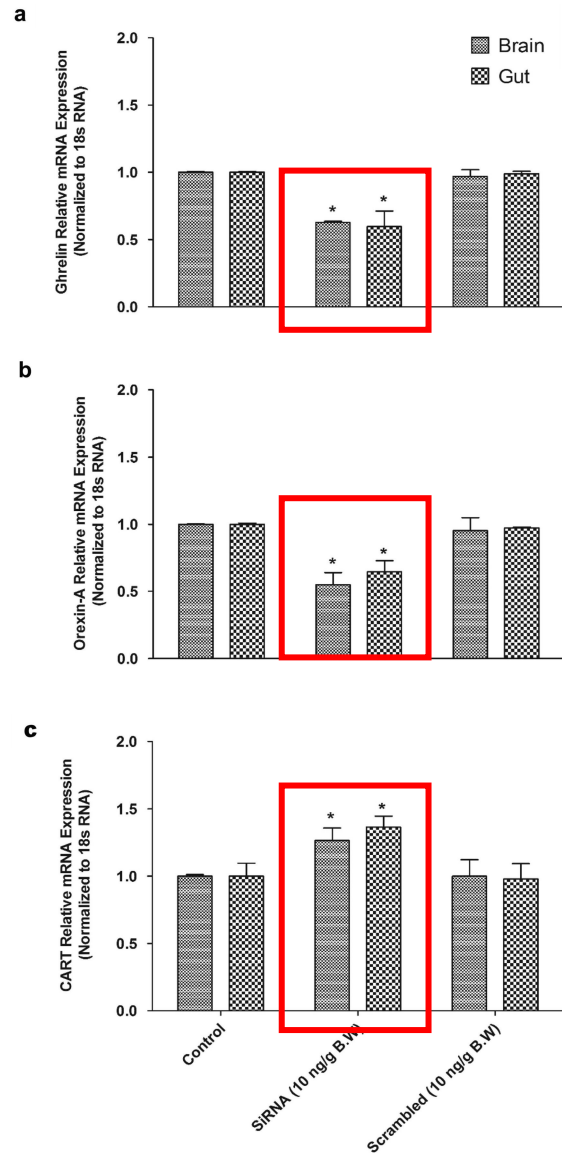
注射Irisin不影响斑马鱼中编码mRNA的食欲调节肽



Intraperitoneal injection **did not affect** ghrelin and orexin-A mRNA expression in zebrafish brain and gut

Irisin **did not affect** CART relative mRNA expression in brain and gut of zebrafish

Irisin敲低导致了orexin的下调和CART的上调



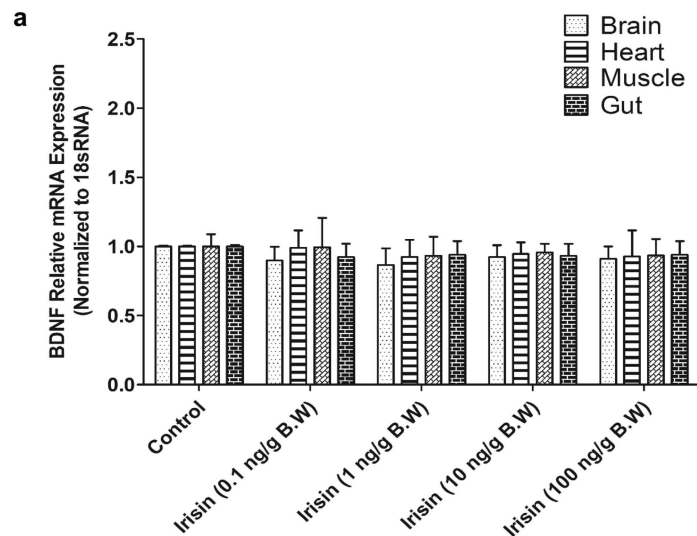
Knockdown of irisin by siRNA on appetite regulatory peptides in zebrafish

Knockdown of irisin downregulated preproghrelin and orexin-A

Knockdown of irisin upregulated CART

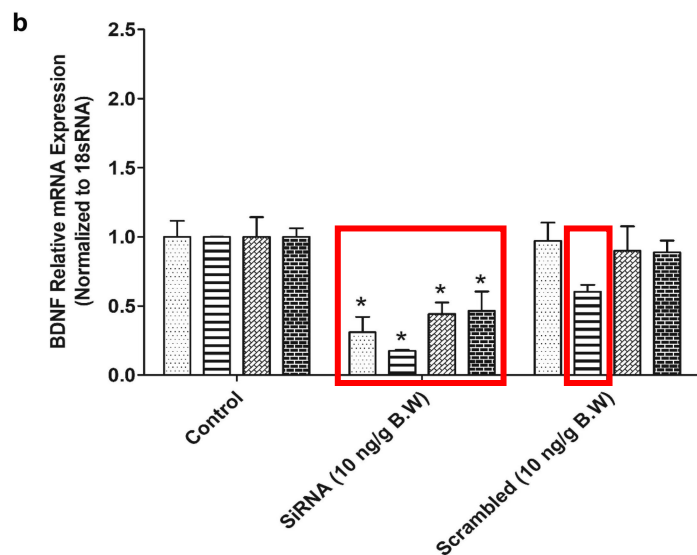


Irisin敲低导致了orexin的下调和CART的上调



BDNF: 脑源性神经营养因子
对神经元的存活、分化、生长发育起重要作用
在哺乳动物中, BDNF调节多巴胺和血清素 (神经递质)
已显示其具有调节饲料摄食和行为的作用

Irisin administration did not affect BDNF relative mRNA expression in zebrafish brain, heart, muscle and gut tissues of zebrafish



Knockdown of irisin (10 ng/g B.W) downregulated BDNF relative mRNA expression in zebrafish when compared to control (saline-injected) group

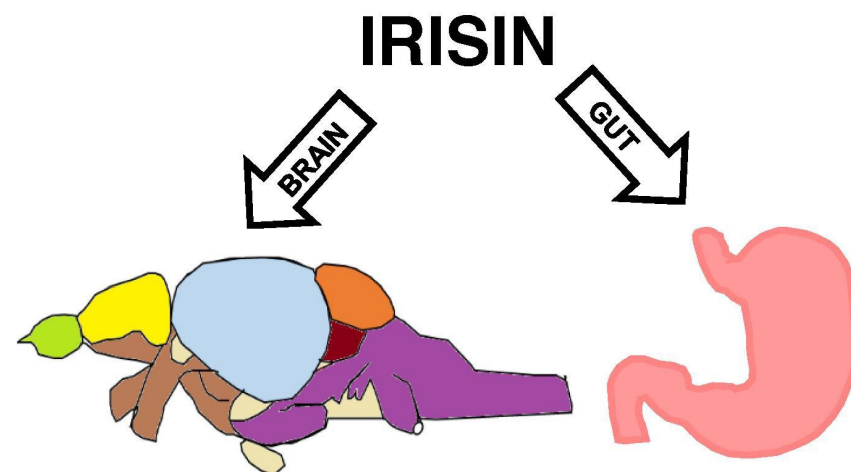


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结论与收获



结论：鸢尾素对食物摄入的影响和斑马鱼食欲调节因子的调节



	Irisin	Irisin siRNA
Feed Intake	↔	↓
Orexin-A, Ghrelin	↔	↓
CART	↔	↑

↑ = Stimulatory effect; ↓ = Inhibitory effect; ↔ = No effects.

结论



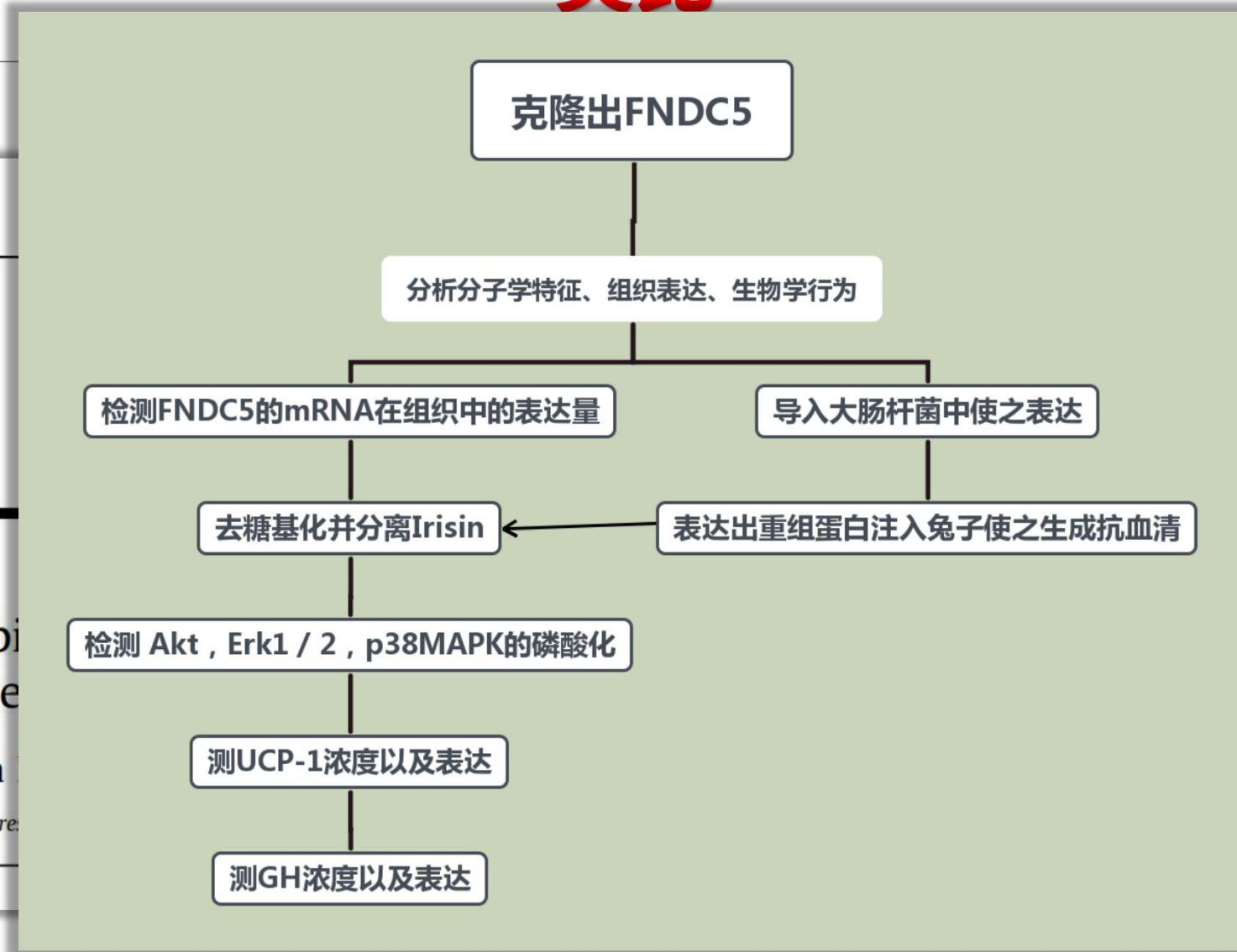
揭示内源性鸢尾素在调节斑马鱼食物摄入中的关键作用

食欲调节肽在鸢尾素的表达和作用中存在物种特异性差异

为今后研究鸢尾素的作用机制及其在斑马鱼其他生理系统中的作用奠定了基础



类比



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Irisin inhibi
pituitary ce

Anji Lian, Xin

Key Laboratory of Bio-re



Irisin对培养的罗非鱼垂体细胞生长激素分泌的抑制作用

读书疑问



While irisin is known to regulate food intake, physical activity and energy homeostasis in mammals (Novelle et al., 2013), its appetite regulatory effects are controversial. In non-obese, non-

food intake

appetite regulatory



读书疑问



... injected with 0.9% sodium chloride (saline). Immediately after the administration of irisin or saline, fish were allowed to recover and were fed a pre-weighed quantity of food, and the food recovered 1 h post-administration was dried overnight at 60 °C, to quantify food intake.

- 1、immediately feed 后为什么在1h内对鱼取样
- 2、此次注射实验是不是对肠道内容物进行取样
- 3、腹腔注射会不会对鱼摄食有影响



读书疑问



由注射irisin实验得出：注射人合成irisin后 zebrafish摄食没有增加。

作者文中解释为

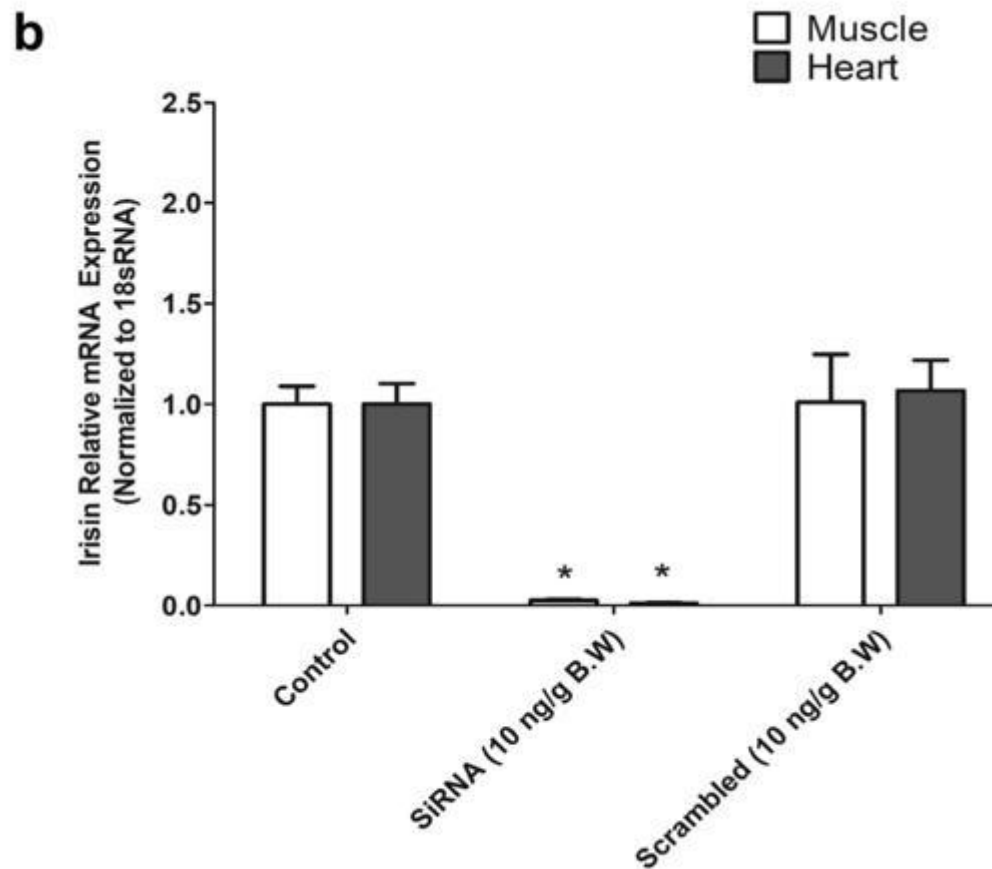
Irisin sequences of zebrafish and humans have 80% amino acid sequence identity.

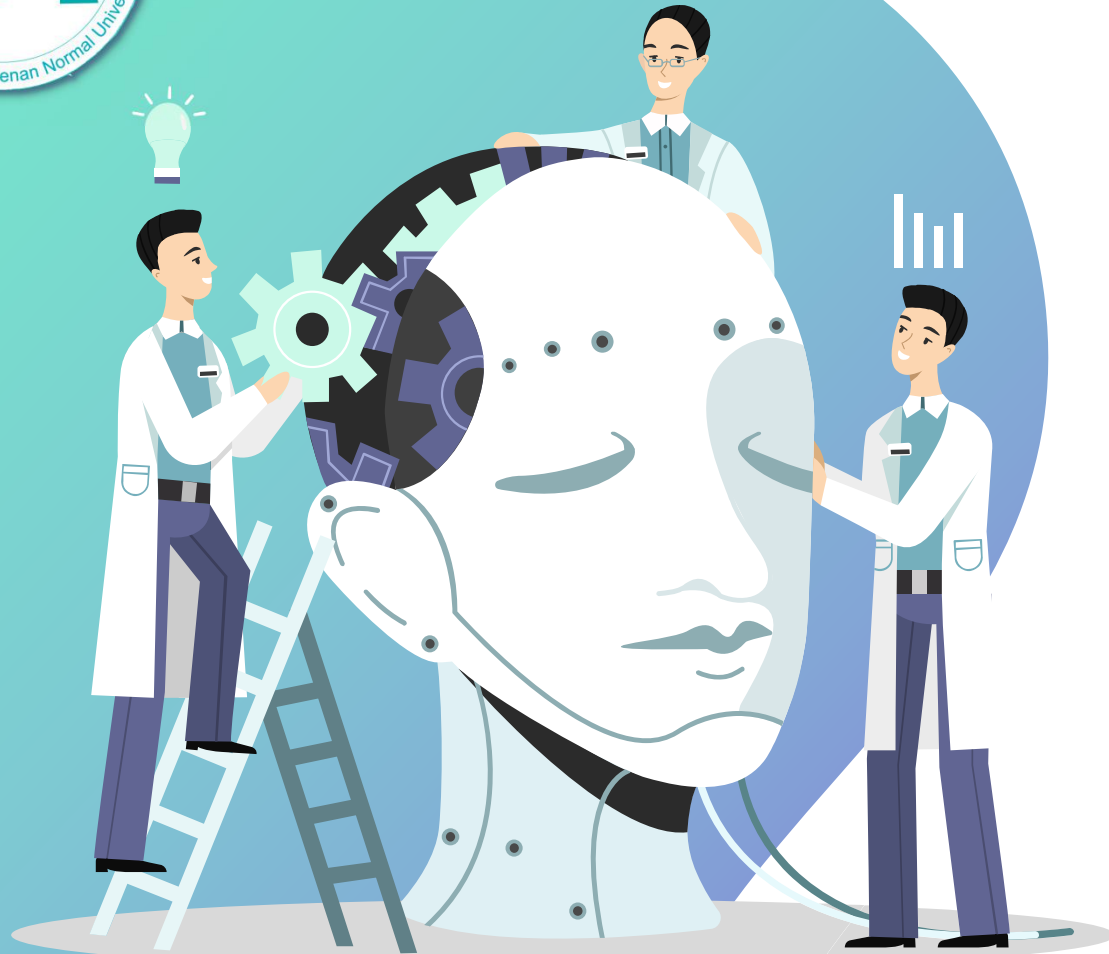
有没有可能是人合成irisin在zebrafish中没有起作用，而siRNA识别了内源irisin。才导致了zebrafish中摄食没有增加。个人认为应该设计纯化zebrafish的irisin，做上述实验更为可靠，如果文中有，为什么要用 human irisin was injected intraperitoneally (i.p.)

源自国坤老师启发，如果是同一个东西，在不同的载体中都可能不表达，那么人Irisin和zebrafish的Irisin可能也有少许不同，这少许不同可能就导致注射的Irisin和注射saline无异。



读书疑问





请各位老师、同学
批评指正!