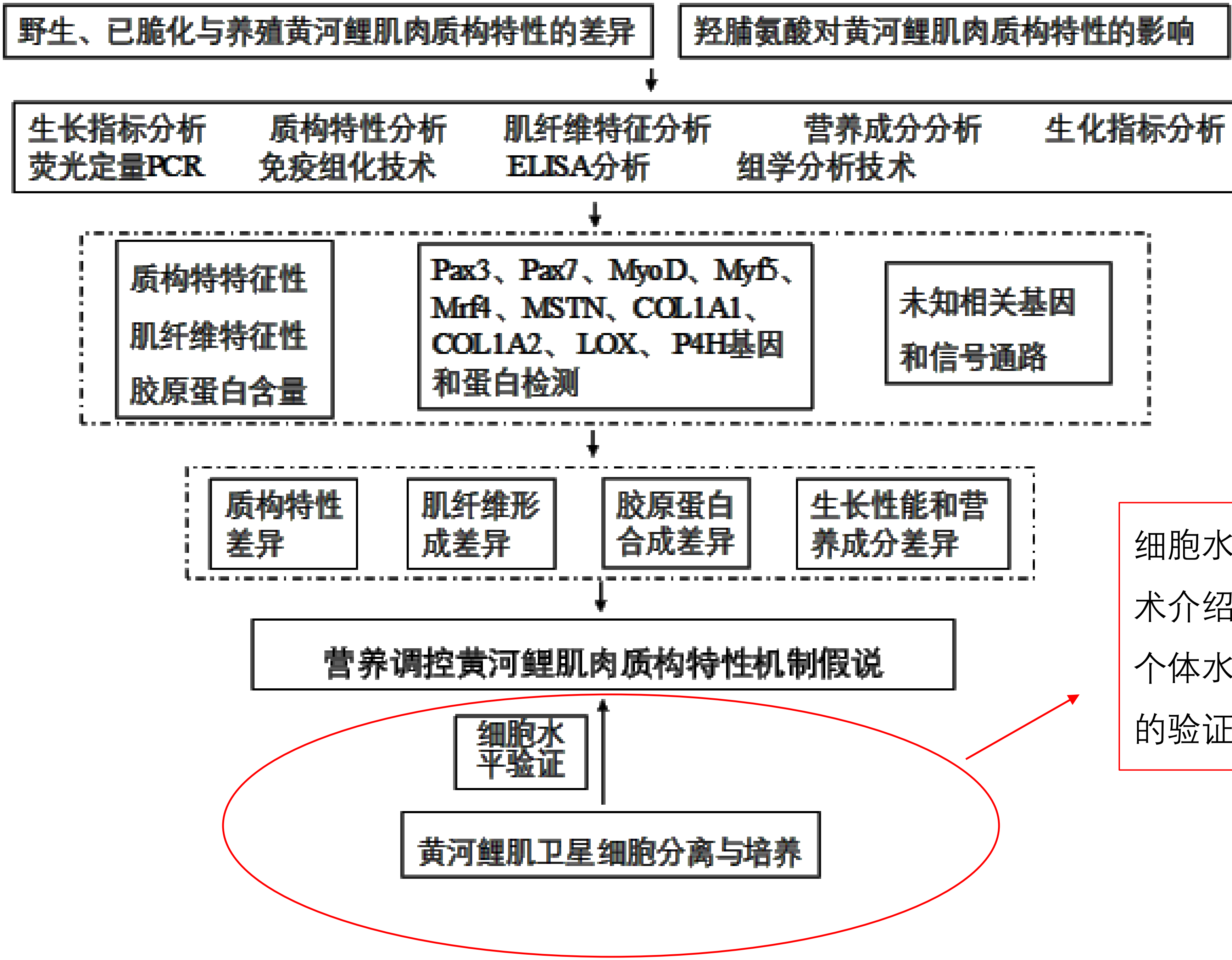
读书报告

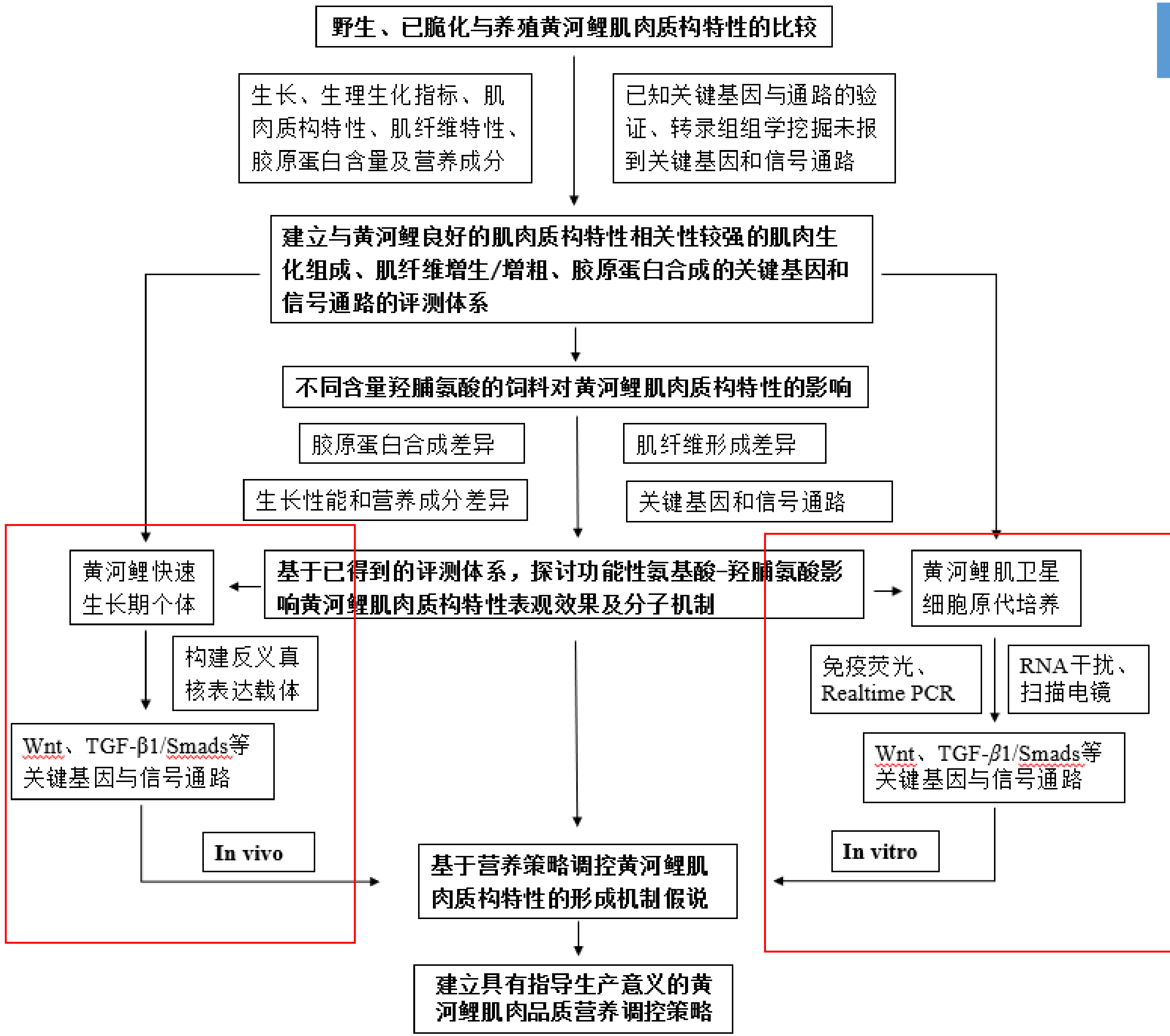
Seminer

河南师范大学 水产动物营养学科研团队

汇报人：宋东蓥 | 2019-11-03



细胞水平具体实
术介绍？
个体水平基因及
的验证？


野生、已驯化与养殖黄河鲤肌肉质构特性的比较
生长、生理生化指标、肌肉质构特性、肌纤维特性、胶原蛋白含量及营养成分
已知关键基因与通路的验证、转录组组学挖掘未报到关键基因和信号通路
建立与黄河鲤良好的肌肉质构特性相关性较强的肌肉生化组成、肌纤维增生/增粗、胶原蛋白合成的关键基因和信号通路的评测体系
不同含量羟脯氨酸的饲料对黄河鲤肌肉质构特性的影响
胶原蛋白合成差异
肌纤维形成差异
生长性能和营养成分差异
关键基因和信号通路
黄河鲤快速生长期个体
基于已得到的评测体系，探讨功能性氨基酸-羟脯氨酸影响黄河鲤肌肉质构特性表观效果及分子机制
黄河鲤肌卫星细胞原代培养
构建反义真核表达载体
免疫荧光、Realtime PCR
RNA干扰、扫描电镜
Wnt、TGF-β1/Smads等关键基因与信号通路
Wnt、TGF-β1/Smads等关键基因与信号通路
In vivo
In vitro
基于营养策略调控黄河鲤肌肉质构特性的形成机制假说
建立具有指导生产意义的黄河鲤肌肉品质营养调控策略

Gene 685 (2019) 32–41

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journal homepage: www.elsevier.com/locate/gene

Research paper

Smad4-dependent regulation of type I collagen expression in the muscle of grass carp fed with faba bean

Er-meng Yu[a,1], Ling-ling Ma[a,b,1], Hong Ji[d], Zhi-fei Li[a], Guang-jun Wang[a], Jun Xie[a,*], De-guang Yu[a], Gen Kaneko[c,*], Jing-jing Tian[a], Kai Zhang[a], Wang-bao Gong[a]

[a] Key Laboratory of Tropical & Subtropical Fishery Resource Application & Cultivation, Pearl River Fisheries Research Institute of CAFS, Xingyu Road No. 1, Guangzhou 510380, China

[b] College of Fisheries and Life, Shanghai Ocean University, Shanghai 201306, China

[c] School of Arts & Sciences, University of Houston-Victoria, Victoria, TX 77901, USA

[d] College of Animal Science and Technology, Northwest A&F University, Yangling 712100, China

TGF-β signalling from cell membrane to nucleus through SMAD proteins

Carl-Henrik Heldin, Kohei Miyazono & Peter ten Dijke

The recent identification of the SMAD family of signal transducer proteins has unravelled the mechanisms by which transforming growth factor-β (TGF-β) signals from the cell membrane to the nucleus. Pathway-restricted SMADs are phosphorylated by specific cell-surface receptors that have serine/threonine kinase activity, then they oligomerize with the common mediator Smad4 and translocate to the nucleus where they direct transcription to effect the cell's response to TGF-β. Inhibitory SMADs have been identified that block the activation of these pathway-restricted SMADs.

基于TGF_Smads信号通路探_省略_豆调节草鱼肌肉_型胶原的分子机制_吕池波	2018/10/5 11:39	CAJ 文件	1,714 KB
草鱼TGF_1_Smad4基因真_省略_A干扰表达载体的构建及其活性验证_张好放	2019/11/2 10:35	CAJ 文件	1,931 KB
草鱼TGF_1_Smad4基因的_省略_过表达和RNA干扰表达载体的构建_张好放	2019/11/2 10:37	Adobe Acrobat ...	4,732 KB
草鱼Smad4基因的克隆_生物信息学分析及反义真核载体的构建_吕池波	2019/11/2 10:38	Adobe Acrobat ...	1,373 KB
草鱼+Sm+ad4基因的克隆、生物信息学分析及反义真核载体的构建	2019/11/2 10:47	Adobe Acrobat ...	4,603 KB
Transcriptional control by the TGF-β Smad signaling system	2019/11/2 12:55	Adobe Acrobat ...	294 KB
The Myofibroblast TGFβ-1, A Conductor which Plays a Key Role in Fibrosis by Reg...	2019/10/29 7:41	Adobe Acrobat ...	573 KB
The materials science of collagen	2019/7/2 17:04	Adobe Acrobat ...	16,360 KB
TGF-β signalling from cell membrane to nucleus through SMAD Proteins	2019/11/2 12:53	Adobe Acrobat ...	309 KB
TGF-β RII真核表达载体及其RNA干扰质粒的构建及鉴定	2019/11/2 10:47	Adobe Acrobat ...	464 KB
TGF_1基因特异性shRNA真核表达载体的构建及其基因抑制作用_尹志康	2019/11/2 10:49	Adobe Acrobat ...	497 KB
Smad4基因siRNA表达载体的构建与鉴定_何云武	2019/11/2 10:49	Adobe Acrobat ...	320 KB
RNA干扰载体活体内耳转染的可行性	2019/11/1 11:15	Adobe Acrobat ...	115 KB
RNAi、miRNA及siRNA的区别和联系	2019/11/1 10:33	Microsoft Word ...	14 KB
Regulation of body mass growth through ctivin type IIB receptor in teleost fish	2019/11/2 12:47	Adobe Acrobat ...	652 KB
Collagen characteristics of farmed Atlantic salmon with firm and soft fillet texture	2019/5/31 19:58	Adobe Acrobat ...	437 KB
Characterization of fish muscle type i collagen	2019/10/24 20:38	Adobe Acrobat ...	477 KB

CONTENTS

1 RESEARCH BACKGROUNDS 研究背景

1 研究背景

RESEARCH BACKGROUNDS

草鱼(*Ctenopharyngodon idella*)是目前全球最大的淡水养殖品种，仅在国内年产量就**500余万**吨（中国渔业统计年鉴，2019年）

1 研究背景

RESEARCH BACKGROUNDS

- ◆ 池塘养殖草鱼肉质品质改良的研究也是营养专家长期关注的焦点
- ◆ 针对目前的草鱼营养学研究现状，谢骏、郁二蒙团队做了一些**营养调控机理**方面的探索

- ◆ 养殖实践中发现摄食**蚕豆**的草鱼肌肉**硬度显著增加**而深受消费者欢迎，研究显示鱼类肌肉硬度的增加与**I型胶原蛋白**的表达有显著相关性，而I型胶原蛋白的表达受 **TGF-β1/Smad4 信号通路**调控

营养调控机理尚不清晰

2

RESEARCH FRAMWORK

理论框架

10

Cryptotanshinone attenuates airway remodeling by inhibiting crosstalk between TWEAK and TGF-β1 signaling pathways in asthma

C Wang, M Zheng, Y Choi, J Jiang, L Li, J Li… - Frontiers in …, 2019 - frontiersin.org

… the level of OVA-specific IgE in BALFs. CTS also inhibited the expressions of α-SMA, TWEAK, Fn14, TGF-β1, Smad4 and phosphorylation of Smad2/3 and STAT3 (Tyr705). In comparison to TWEAK inhibitor or TWEAK siRNA …

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11

95

12

Correlation between S100A11 and the TGF-β_1/SMAD4 pathway and its effects on the proliferation and apoptosis of pancreatic cancer cell line PANC-1

YF Ji, T Li, F Jiang, WK Ni, CQ Guan, ZX Liu… - Molecular and cellular …, 2019 - Springer

… Effects of TGF-β1-siRNA interference on TGF-β1, SMAD4, P21 WAF1 , S100A11 protein, and mRNA expression in PANC-1 cells … b TGF-β1, SMAD4, P21 WAF1 , and S100A11 mRNA expressions were determined by quantitative polymerase chain reaction (qPCR) …

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Magnesium isoglycyrrhizinate ameliorates high fructose-induced liver fibrosis in rat by increasing miR-375-3p to suppress JAK2/STAT3 pathway and TGF-β1/Smad …

Y Yang, X Zhao, H Xu, S Wang, Y Pan… - Acta Pharmacologica …, 2019 - nature.com

Article.

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15

91

16

111

MiR-146a attenuates liver fibrosis by inhibiting transforming growth factor-β1 mediated epithelial-mesenchymal transition in hepatocytes

Y Zou, S Li, Z Li, D Song, S Zhang, Q Yao - Cellular signalling, 2019 - Elsevier

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51

18

INHBA gene silencing inhibits gastric cancer cell migration and invasion by impeding activation of the TGF-β signaling pathway

ZL Chen, L Qin, XB Peng, Y Hu… - Journal of cellular …, 2019 - Wiley Online Library

… RT-qPCR and western blot analysis were applied in order to determine the mRNA and protein expression of INHBA, TGF-β1, Smad4, VEGF, AKT, Smad7, and PTEN and phosphorylated smad2, smad3, and AKT proteins in the GC tissues, and the results (Figure 3a–c …

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19

73

20

Smad4-dependent regulation of type I collagen expression in the muscle of grass carp fed with faba bean

E Yu, L Ma, H Ji, Z Li, G Wang, J Xie, D Yu, G Kaneko… - Gene, 2019 - Elsevier

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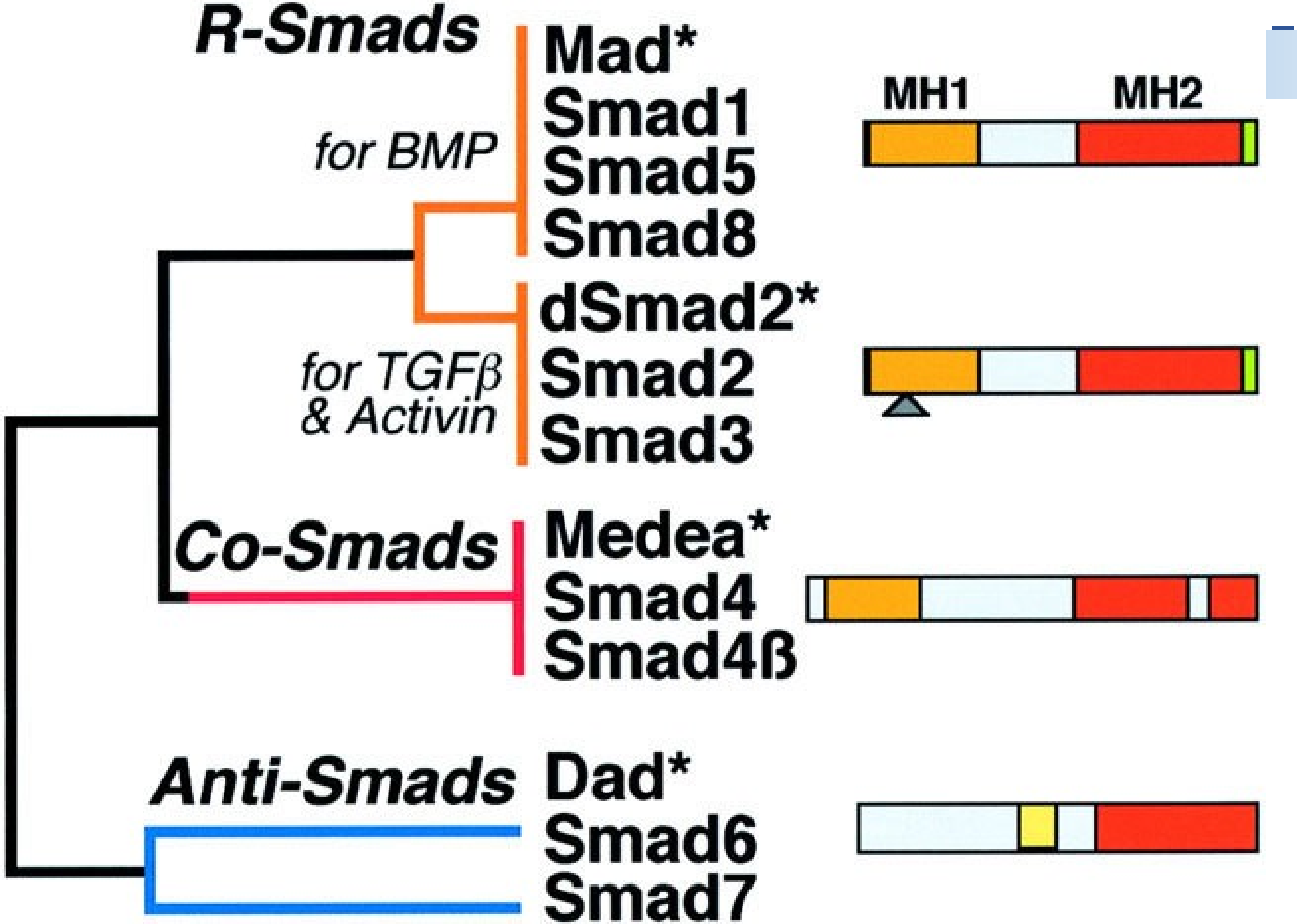
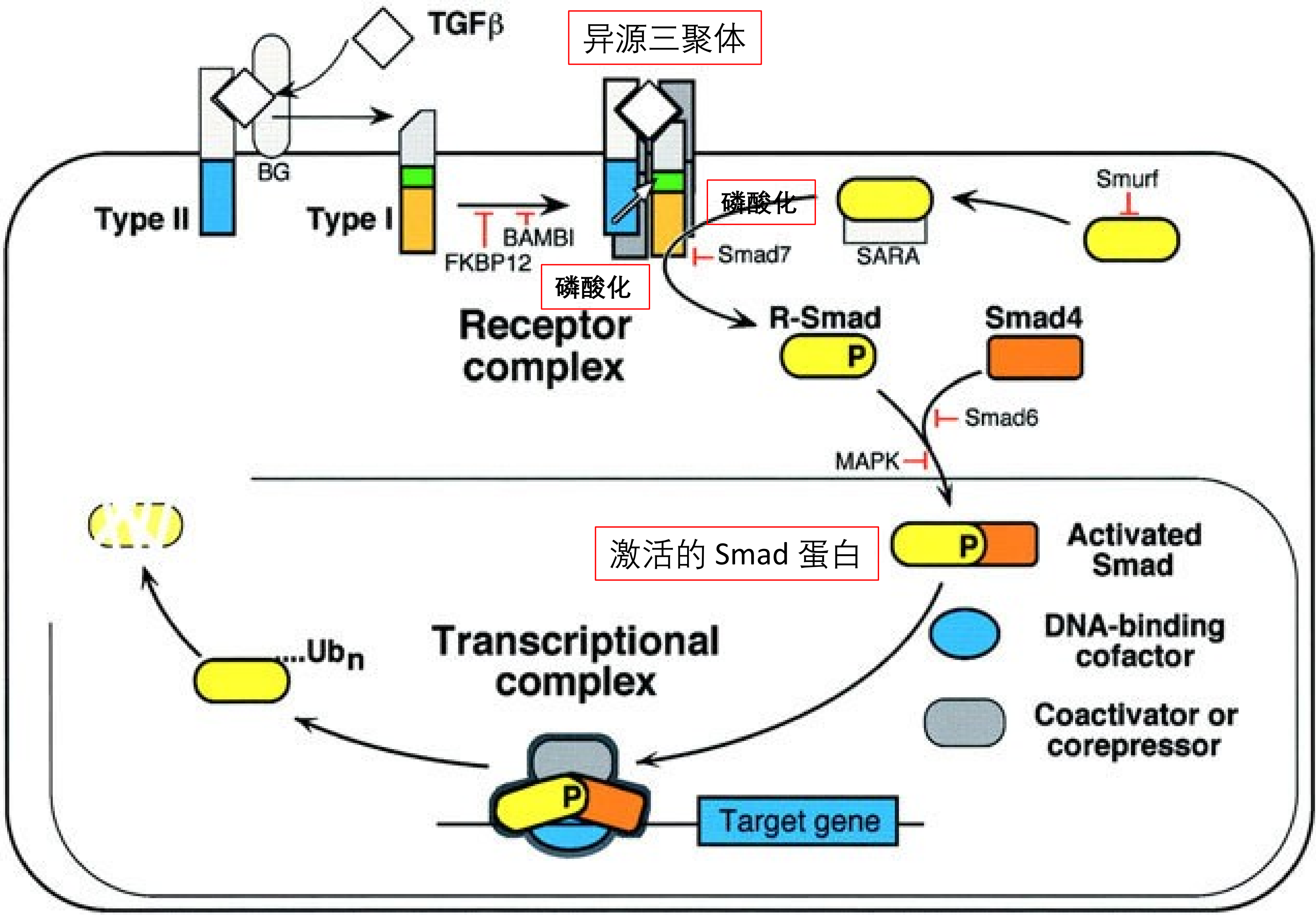
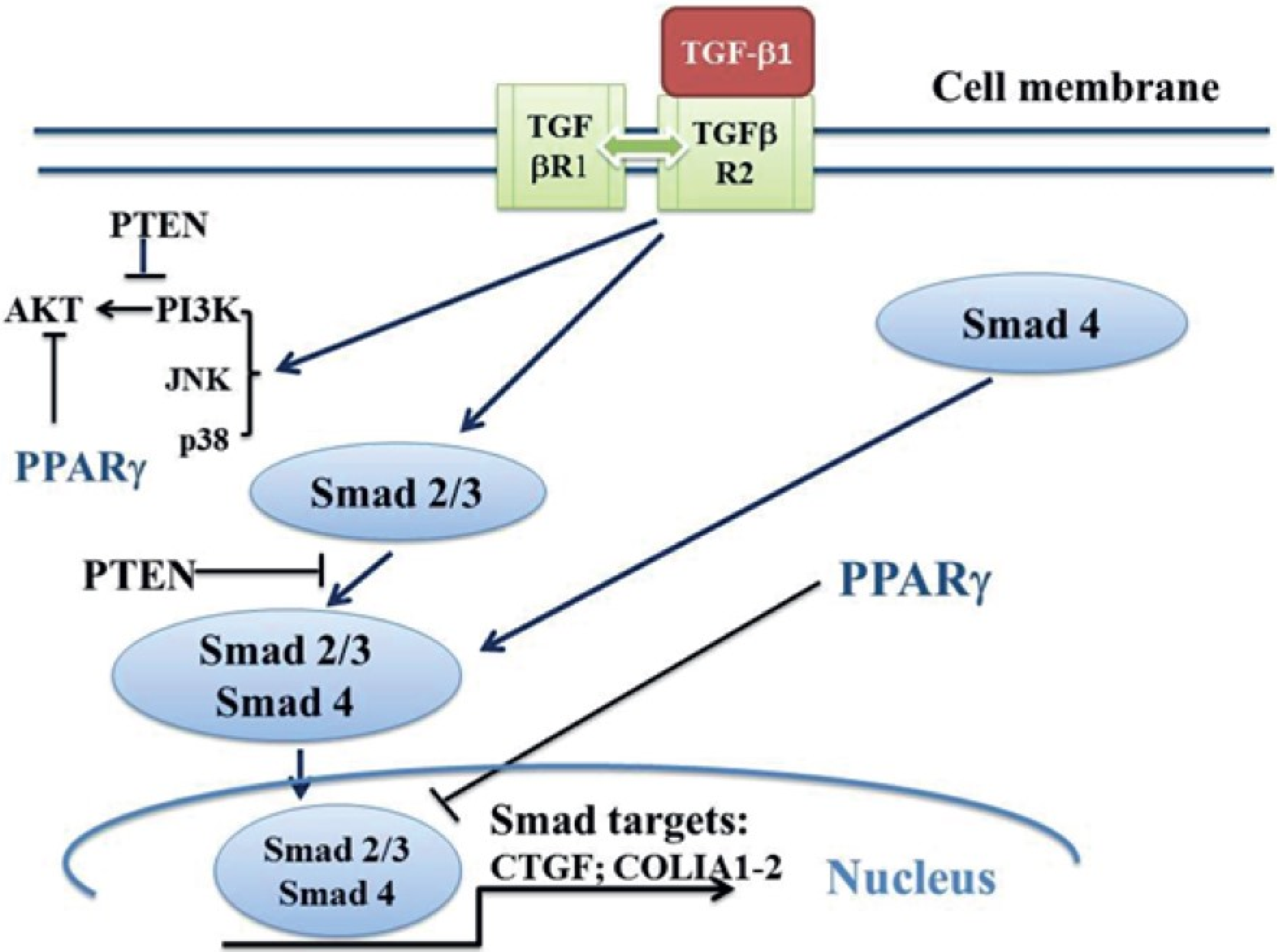
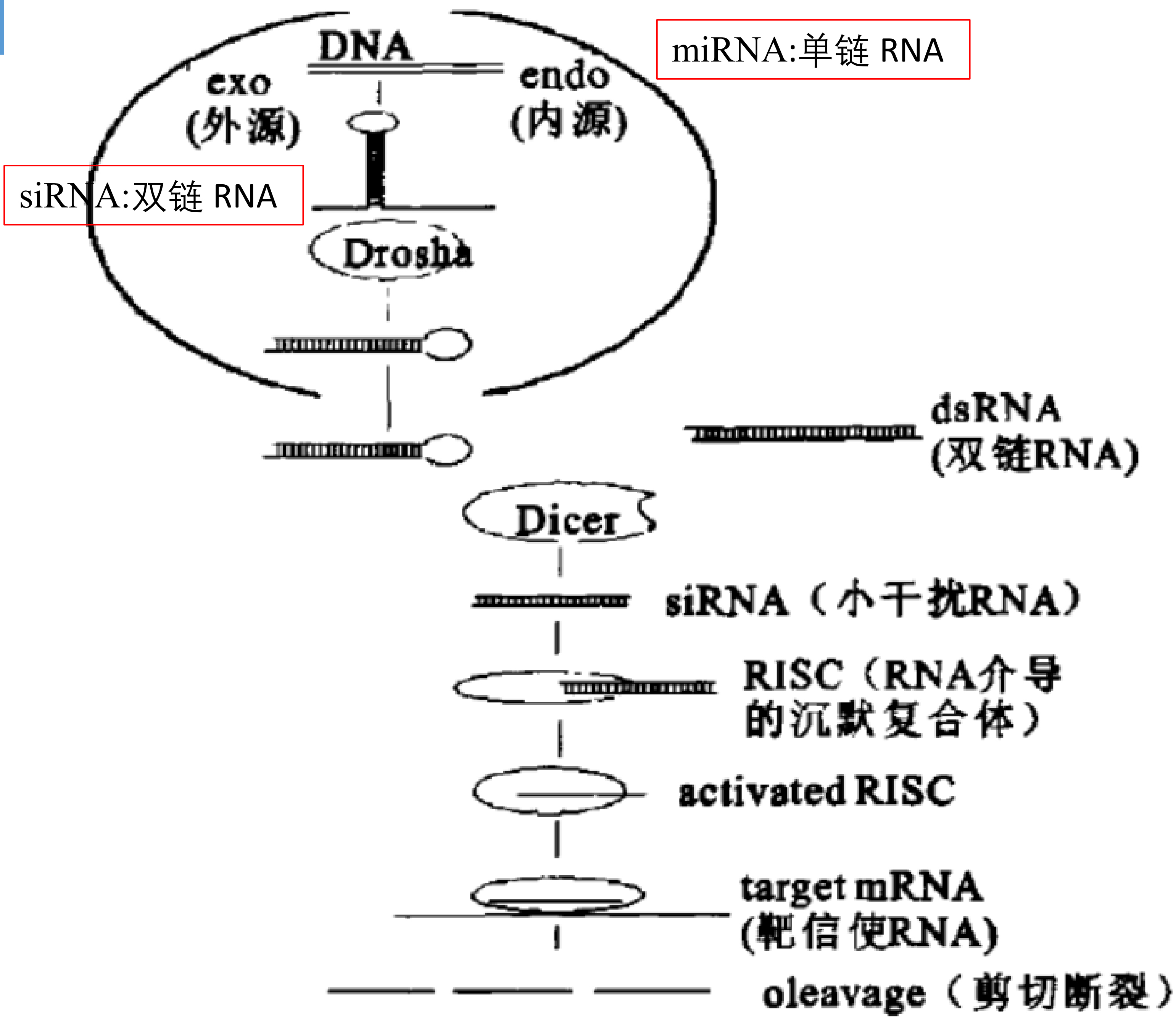

R-Smads
for BMP
Mad*
Smad1
Smad5
Smad8
for TGFβ
& Activin
dSmad2*
Smad2
Smad3
Co-Smads
Medea*
Smad4
Smad4ß
Anti-Smads
Dad*
Smad6
Smad7
MH1
MH2



TGFβ
异源三聚体
BG
Type II
Type I
磷酸化
BAMBI
FKBP12
磷酸化
Smad7
SARA
Smurf
Receptor complex
R-Smad
P
Smad4
Smad6
MAPK
激活的 Smad 蛋白
P
Activated Smad
DNA-binding cofactor
Coactivator or corepressor
...Ubn
Transcriptional complex
P
Target gene


TGF-β1
Cell membrane
TGF βR1
TGFβ R2
PTEN
AKT
PI3K
JNK
p38
PPARγ
Smad 4
Smad 2/3
PTEN
PPARγ
Smad 2/3 Smad 4
Smad targets:
CTGF; COLIA1-2
Smad 2/3 Smad 4
Nucleus

2

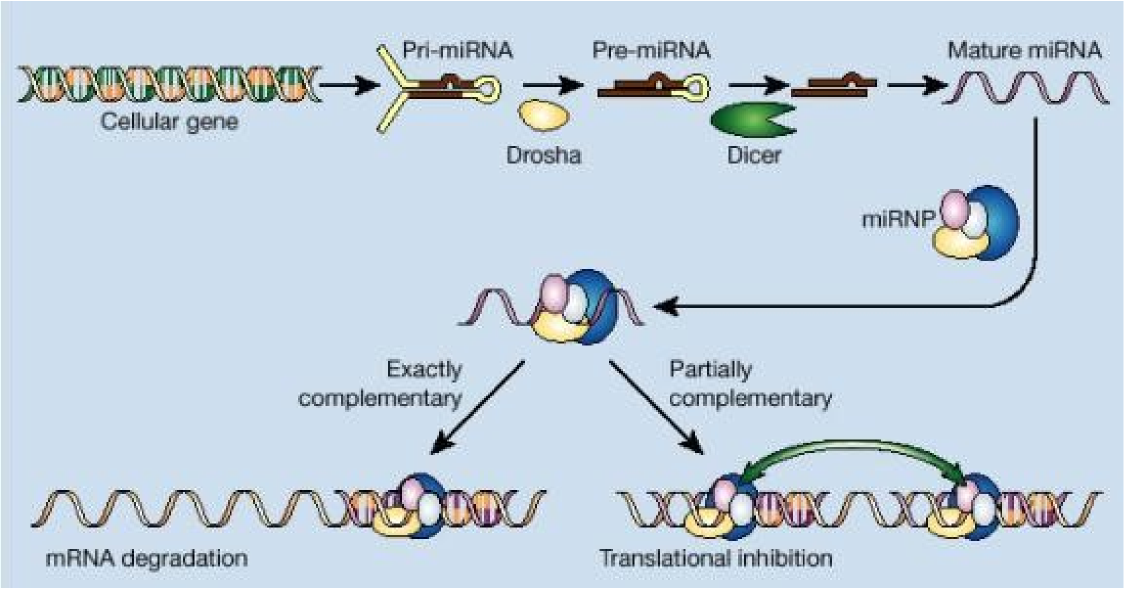

miRNA:单链 RNA
siRNA:双链 RNA
DNA
exo
(外源)
endo
(内源)
Drosha
dsRNA
(双链RNA)
Dicer
siRNA（小干扰RNA）
RISC（RNA介导
的沉默复合体）
activated RISC
target mRNA
(靶信使RNA)
oleavage（剪切断裂）

RNAi机制的缺陷

RNAi存在技术其核心内容是siRNA能够抑制同源基因的表达。对于siRNA而言，现在颇有争议的地方在于它对靶基因的沉默特异性，主要体现在两个方面

（1）打破内源性miRNA的平衡：非特异性表型的缺失或缺陷和毒理效应

（2）特异的转录后沉默有效性：脱靶效应



3 RESEARCH METHODS 研究方法

3 研究方法

RESEARCH METHODS

为了探究草鱼肌肉品质改良的营养调控机制，郁二蒙等主要构建了草鱼TGF-β1/Smad4信号通路关键信号因子*TGF-β1*和*Smad4*基因的**真核过表达载体**pcDNA3.1 (+)-TGF-β1 和 pcDNA3.1(+)-Smad4 **和 RNA 干扰表达载体** pRNA-6.1/Neo-TGF-β1（I）、pRNA-6.1/Neo-TGF-β1(II)、pRNA -6.1/Neo -TGF-β1(III)、pRNA-6.1/Neo-Smad4(I)、pRNA-6.1/Neo-Smad4(II)和 pRNA-6.1/ Neo-Smad4 (III)，随后分别在**细胞**和**鱼体水平**验证构建的过表达和 RNA 干扰表达载体的活性，继而探索鱼类肌肉硬度的增加与I型胶原蛋白的表达的**调控机理。**

（1）养殖实验

（2）*TGF-β1*、*Smad4* 基因克隆及真核过表达和 RNA 干扰表达载体的构建

（3）*TGF-β1*、*Smad4* 基因过表达和 RNA 干扰表达载体细胞水平和在体水平的活性验证。

500 ± 50 g

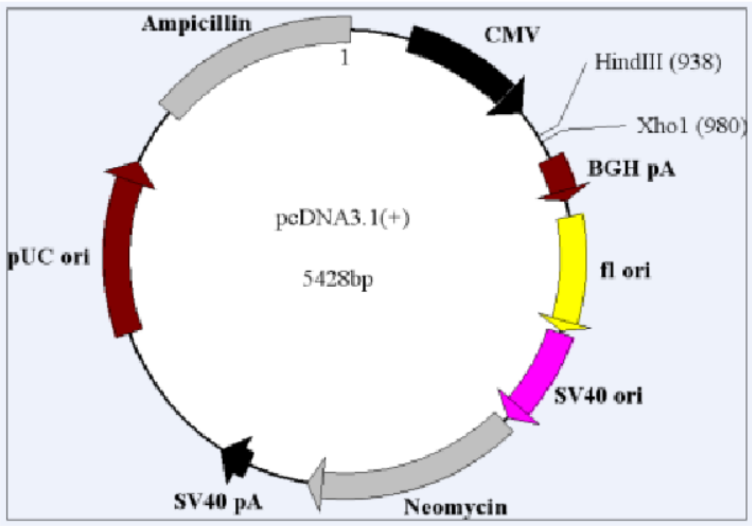
2.5×2.5×1.2m水泥池

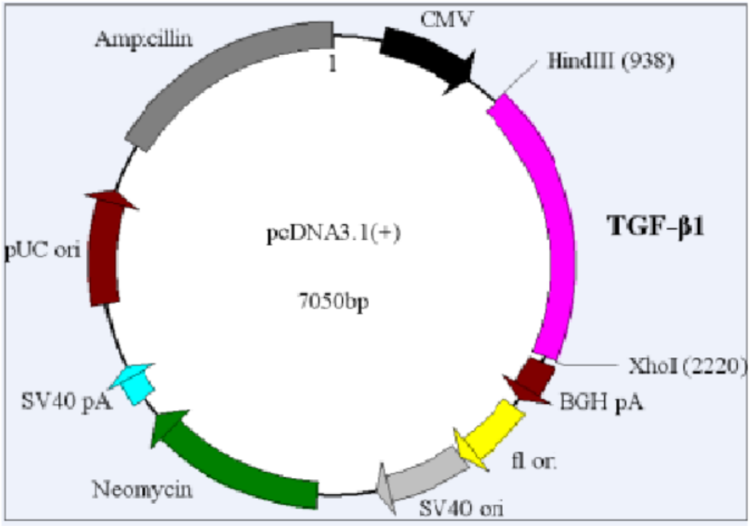
8:00, 14:00 and 18:00

投饲量4%蚕豆pre-soaked

Sampled：0 (control; before the initiation of faba bean feeding), 30, 60, 90 and 120 days

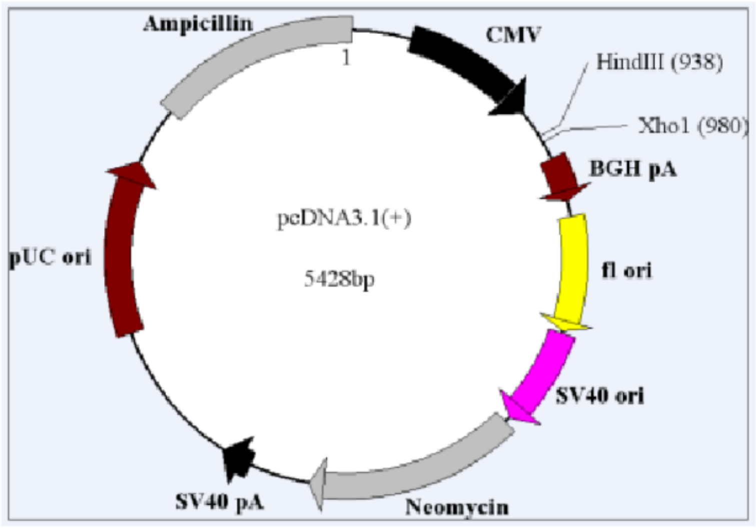
TGF-β1 基因的过表达载体构建

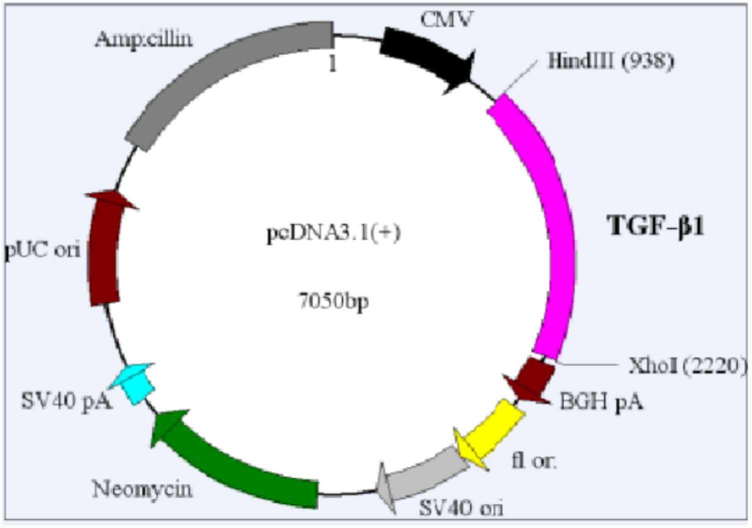




TGF-β1 基因 ORF 片段正向插入表达载体

TGF-β1 基因的过表达载体构建





TGF-β1 基因 ORF 片段正向插入表达载体

TGF-β1、*Smad4* 基因 RNA 干扰表达载体的构建

shRNA 靶序列的设计

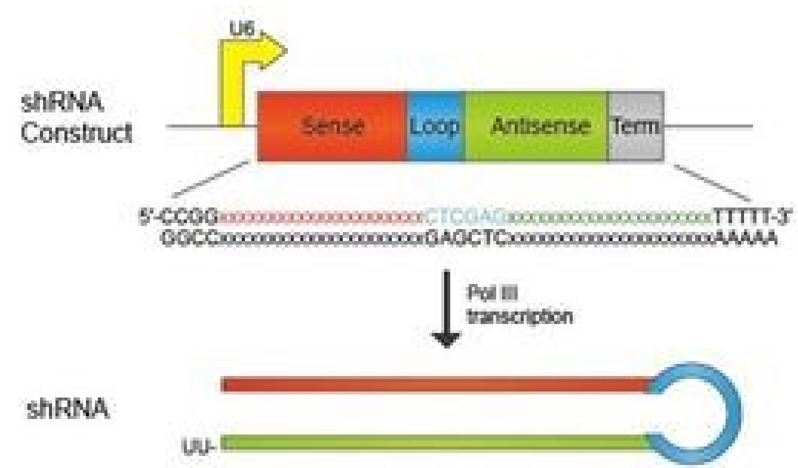
①从 *TGF-β1*、*Smad4* 基因 ORF 序列起始密码子 AUG 开始搜寻下游 19 个碱基长度且 GC 含量约在 50%左右的正反义互补序列 3 对；

②将每一对正反义序列展开，在中间插入 10 个碱基的 Loop 片段；

③为确保转录能够终止，在每一条序列的 3′端加上 6 个连续的 T 碱基外加两个胸腺嘧啶，补加胸腺嘧啶是使人工合成的干扰片段尽量接近于自然形成的 siRNA，更有利于启动双链 RNA 的沉默效应；

④在每对序列两端加上相应的酶切位点，根据干扰载体图谱，选取的酶切位点，这样转录形成的整条 RNA 即为 shRNA;

shRNA（short hairpin） 的合成



Loop 片段

TGF-β1、*Smad4* 基因 RNA 干扰表达载体的构建

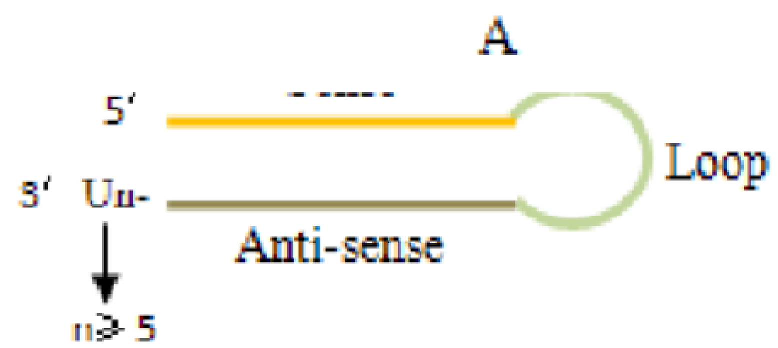
双链 shRNA 的制备

①相应的正义和反义链，退火形成双链shRNA 茎环样双链结构；

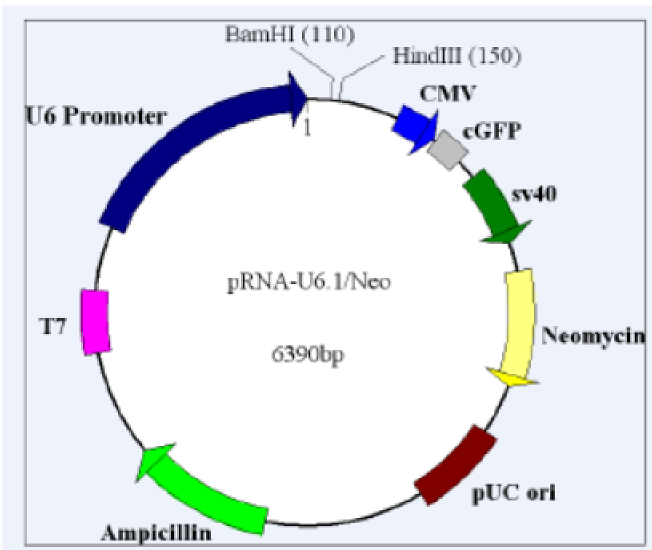
②shRNA 发挥效应时会在细胞内 Dicer 酶作用下经剪接形成只包含 19 个碱基长度的双链小 RNA 即 dsRNA, dsRNA 在解旋酶作用下解开双链同时与胞内核酶复合物相结合，进而形成RNA 沉默诱导复合物 RISC

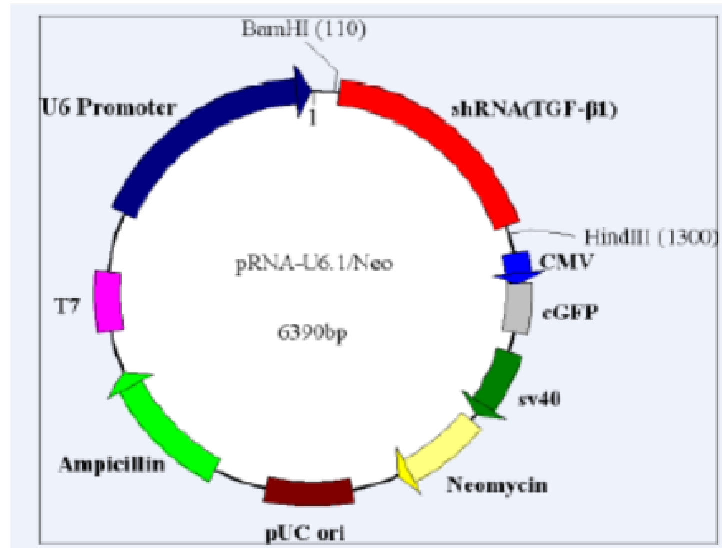
③激活的 RISC 可根据碱基互补配对原则结合到同源的 m RNA 转录本上 ， 最后在核酸酶作用下将 mRNA 彻底降解，从而实现对目的基因的精确沉默

shRNA（short hairpin） 的合成



TGF-β1 基因 shRNA 片段插入干扰表达载体





F1: GATCCCGCAAGCTACGACTGCCTAATTGATATCCGTTAGGCAGTCGTAGCTTGCTTTTTTCCAAA

R1: AGCTTTTGGAAAAAAGCAAGCTACGACTGCCTAACGGATATCAATTAGGCAGTCGTAGCTTGCGG

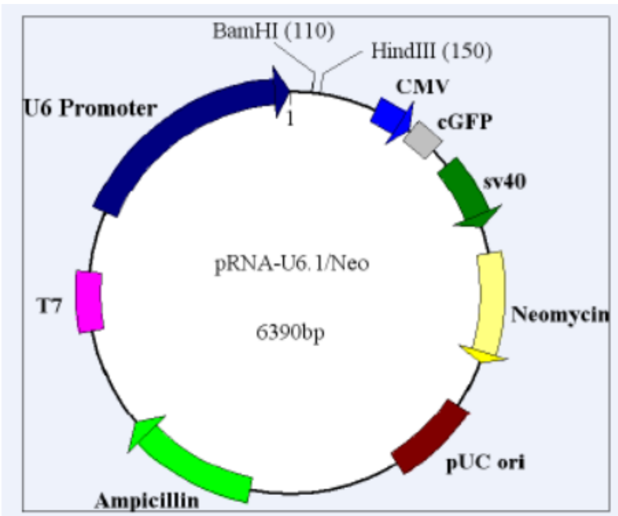
F2: GATCCCGCATGCACATATGGTTCAATTGATATCCGTTGAACCATATGTGCATGCTTTTTTCCAAA

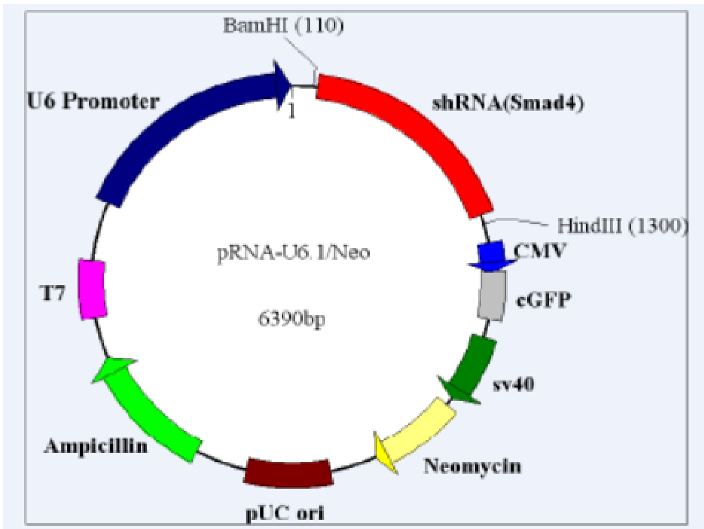
R2: AGCTTTTGGAAAAAAGCATGCACATATGGTTCAACGGATATCAATTGAACCATATGTGCATGCGG

F3: GATCCCGCAGGTTTAGCTGTATCATTTGATATCCGATGATACAGCTAAACCTGCTTTTTTCCAAA

R3: AGCTTTTGGAAAAAAGCAGGTTTAGCTGTATCATCGGATATCAAATGATACAGCTAAACCTGCGG

Smad4 基因shRNA 片段插入干扰表达载体





F1′ ：GATCCCGCCAGCAAATGTGTGACCAT<u>TTGATATCCG</u>ATGGTCACACATTTGCTGGTTTTTCCAAA

R1′ ：AGCTTTTGGAAAAACCAGCAAATGTGTGACCAT<u>CGGATATCAA</u>ATGGTCACACATTTGCTGGCGG

F2′ ：GATCCCGCCATCAGAACGGCCATCTT<u>TTGATATCCG</u>AAGATGGCCGTTCTGATGGTTTTTCCAAA

R2′ ：AGCTTTTGGAAAAACCATCAGAACGGCCATCTT<u>CGGATATCAA</u>AAGATGGCCGTTCTGATGGCGG

F3′ ：GATCCCGCATTTCAGCCACCGATAT<u>TTGATATCCG</u>ATATCGGTGGCTGAAATGCTTTTTCCAAA

R3′ ：AGCTTTTGGAAAAAGCATTTCAGCCACCGATAT<u>CGGATATCAA</u>ATATCGGTGGCTGAAATGCGG

过表达和 RNA 干扰表达载体细胞水平活性检测

草鱼成纤维细胞的分离培养 → 斑马鱼 ZF4 细胞瞬时转染 → 293T 细胞（人胚肾上皮细胞）

↓

36h后，荧光定量检测 *TGF-β1*、*COL1-A1*、*COL1-A2*

过表达和 RNA 干扰表达载体鱼体活性检测

过表达实验组

RNA 干扰表达实验组

阴性对照组

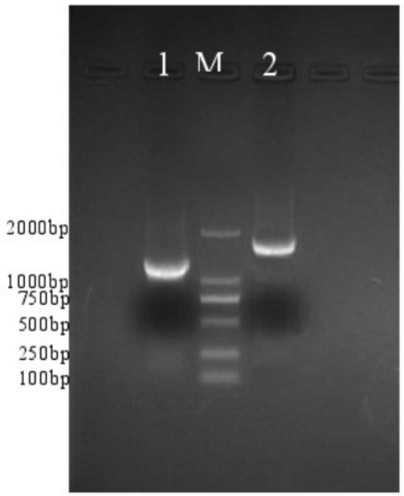
载体注射

注射后1 d, 2 d, 5 d, 10 d 和 15 d 取样，WB及荧光定量检测 *TGF-β1*、*COL1-A1*、*COL1-A2*及相应的基因

4 ANALYSIS AND DISCUSSION 分析与讨论

4 分析与讨论

ANALYSIS AND DISCUSSION

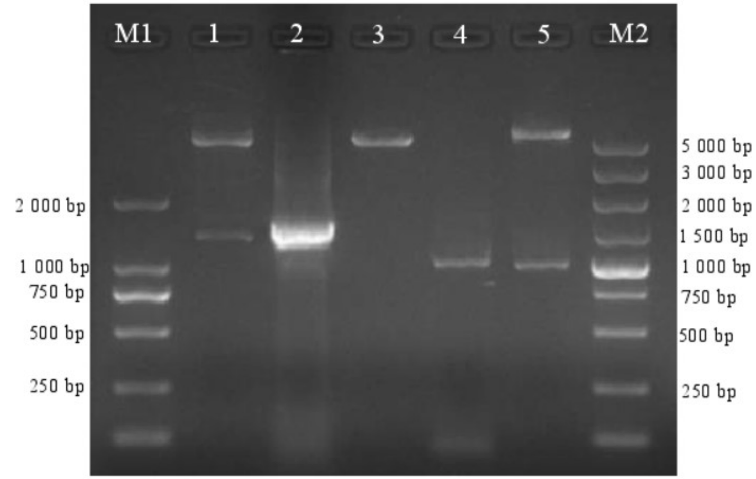


TGF-β1、*Smad4* 基因 ORF 片段 PCR 结果检测

1: 以 c DNA 为模板扩增的 *TGF-β1* 基因 ORF 片段,

M：DNA marker DL2000,

2：以cDNA
为模板扩增的 *Smad4* 基因 ORF 片段



TGF-β1、*Smad4* 重组载体双酶切鉴定

M1: DNA marker DL2000,

1: pcDNA3.1(+)-*Smad4* 重组载体双酶切,

2: *Smad4* 基因 ORF 片段,

3: pcDNA3.1(+)双酶切,

4: TGF-β1 基因 ORF 片段,

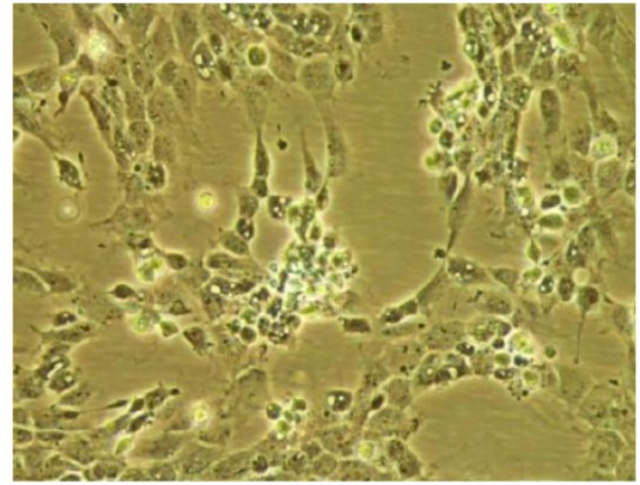
5: pcDNA3.1(+)-*TGF-β1* 重组载体双酶切,

M2: DNA marker DL5000

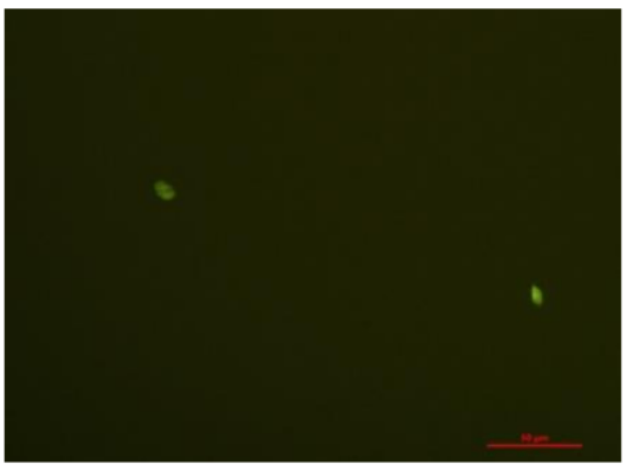
4 分析与讨论 ANALYSIS AND DISCUSSION

草鱼成纤维细胞培养及ZF4细胞转染

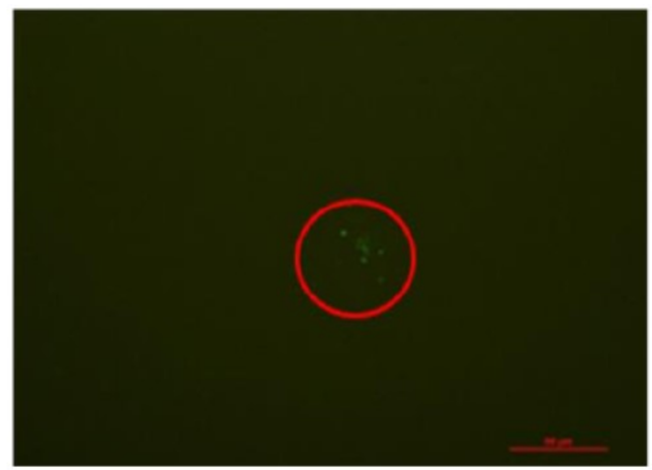
A、草鱼成纤维细胞形态图



B、草鱼成纤维细胞低密度伸展状态

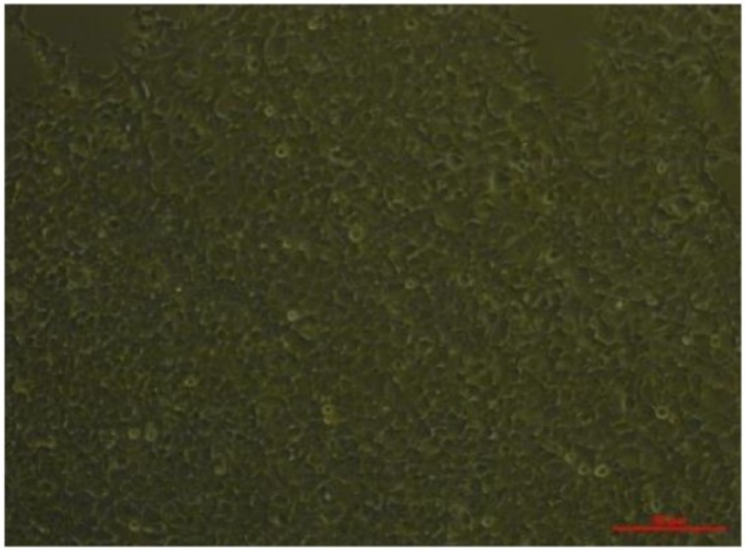


C、ZF4细胞转染效果

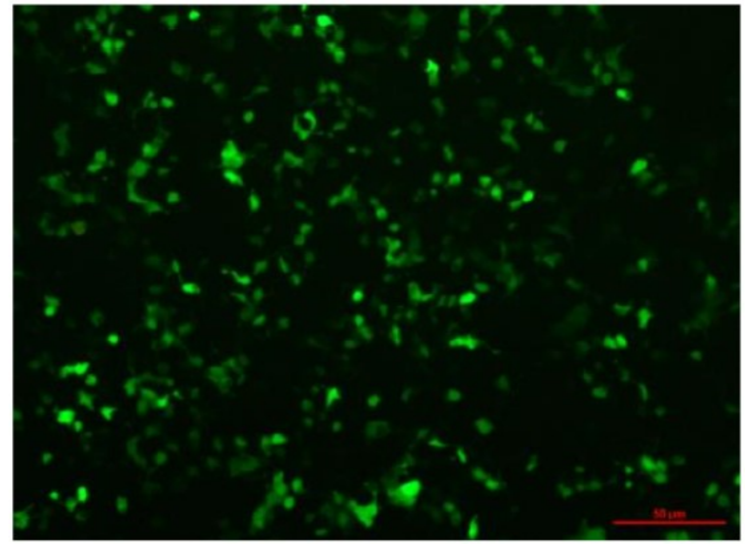


D、ZF4细胞转染效果

293T细胞形态和转染效果图



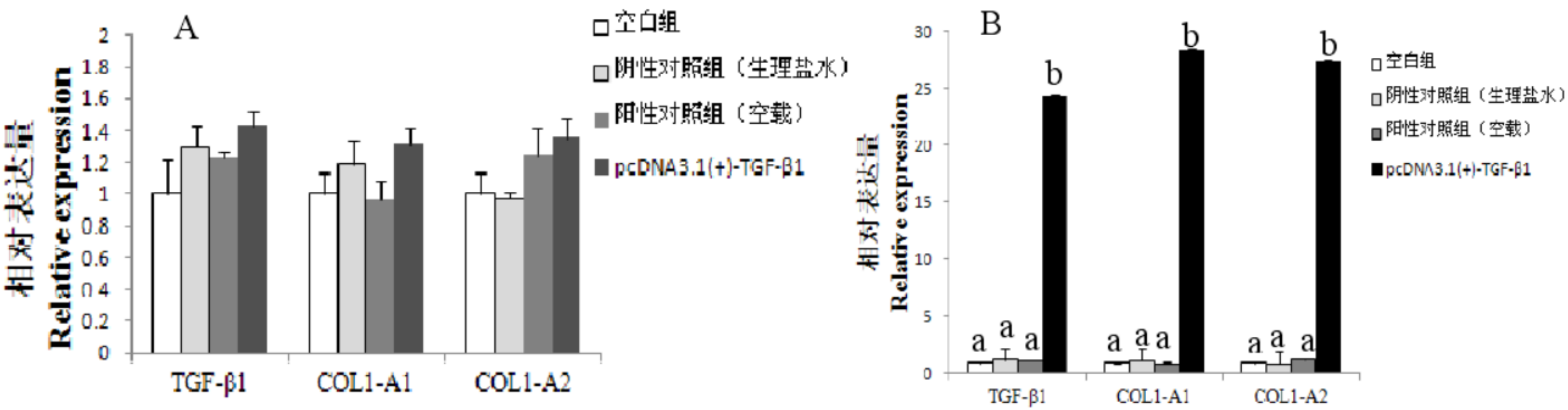
A．293T 细胞形态图



B．293T 细胞转染效果图

A 图展示了该细胞系形态细胞呈扁平圆形，团团粘附在一起，增殖十分迅速；B 图为该细胞系经转染后绿色荧光蛋白检测情况，可以看到转染效率很高，最高可达到 80%。

4 分析与讨论 ANALYSIS AND DISCUSSION

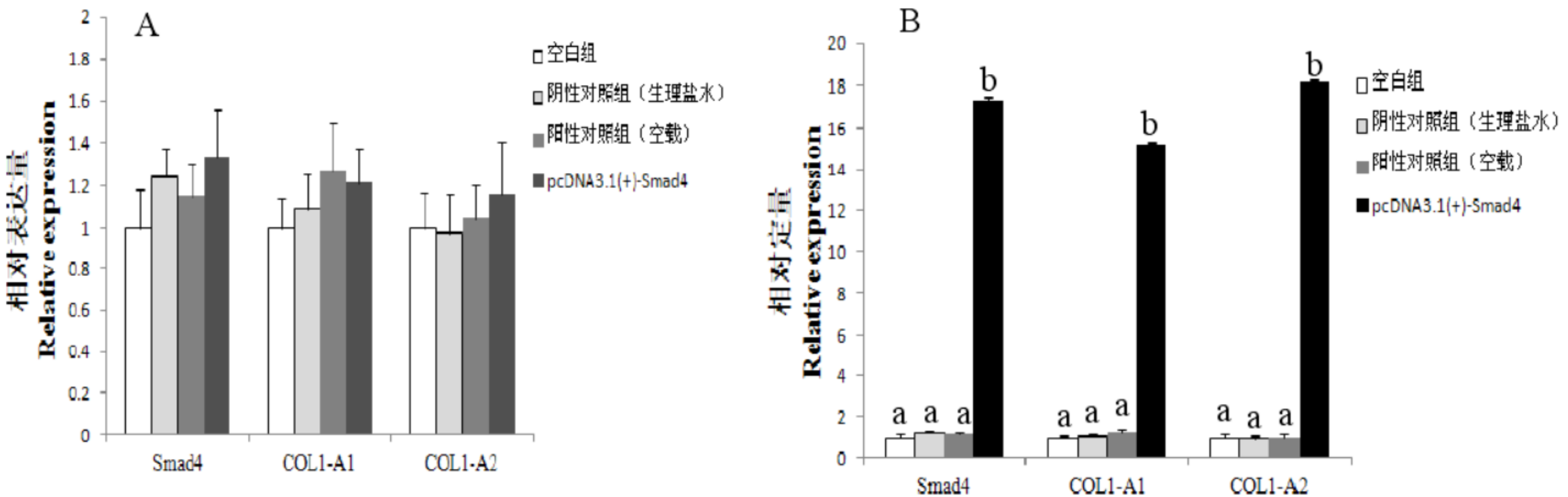


单独转染 pcDNA3.1(+)-*TGF-β1* 过表达载体 36h 后的相对定量

A 显示的是人*TGF-β1,COL1-A1,COL1-A2* 三种基因空白组与对照组的相对表达量，

B 显示的是草鱼*TGF-β1,COL1-A1,COL1-A2* 三种基因的相对表达量，不同字母的代表相互比较具显著性差异（$P<0.05$）

4 分析与讨论 ANALYSIS AND DISCUSSION

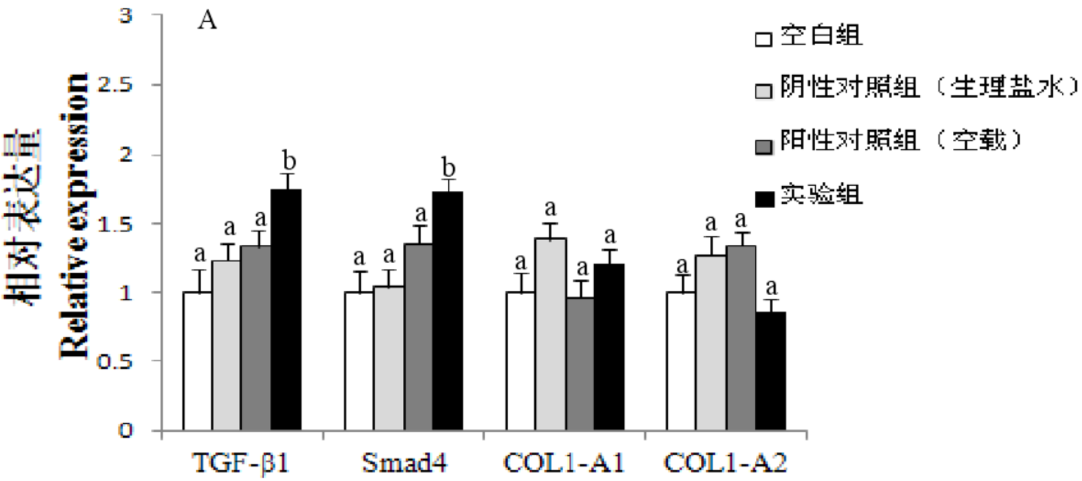


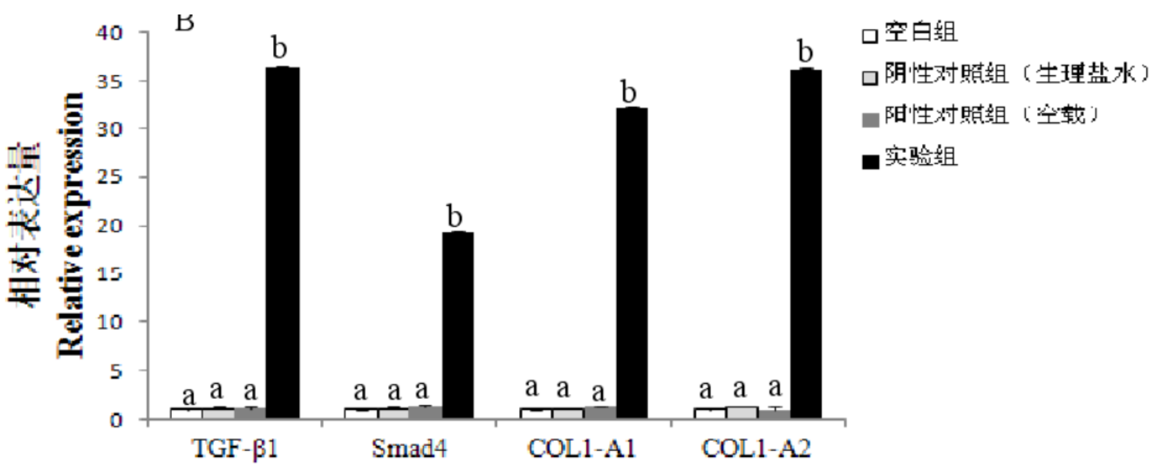
单独转染 pcDNA3.1(+)-*Smad4* 过表达载体 36h 后的相对定量

A 显示的是人*Smad4,COL1-A1,COL1-A2* 三种基因空白组与对照组的相对表达量

B 显示的是草鱼*Smad4,COL1-A1,COL1-A2* 三种基因的相对表达量，不同字母代表相互之间具显著性差异（P<0.05）

4 分析与讨论 ANALYSIS AND DISCUSSION



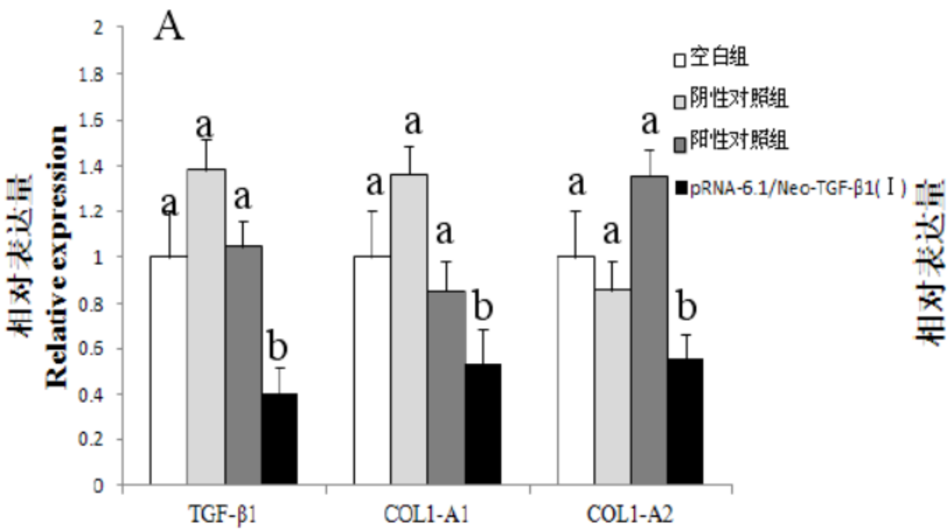


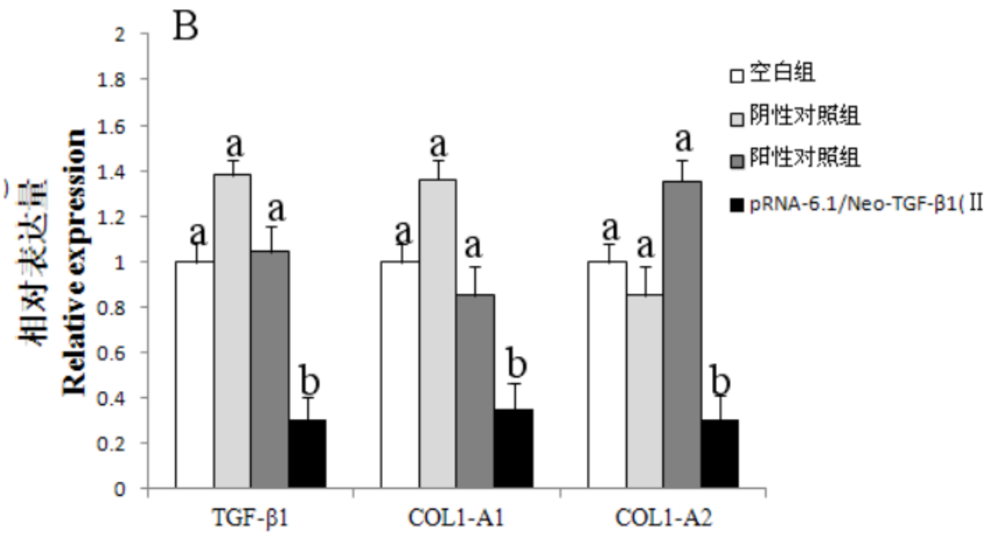
同时转染 pcDNA3.1(+)-*TGF-β1*, pcDNA3.1(+)-*Smad4* 过表达载体 36h 后的相对定量

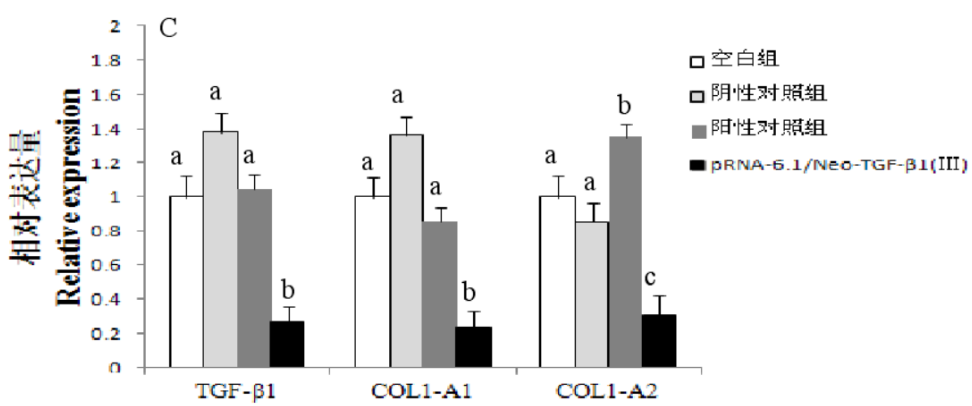
A显示的是人 *TGF-β1, Smad4, COL1-A1, COL1-A2* 四种基因的相对表达量,

B 显示的是草鱼*TGF-β1, Smad4, COL1-A1, COL1-A2* 四种基因的相对表达量,不同字母代表相互之间存在显著性差异（$P<0.05$）

4 分析与讨论 ANALYSIS AND DISCUSSION



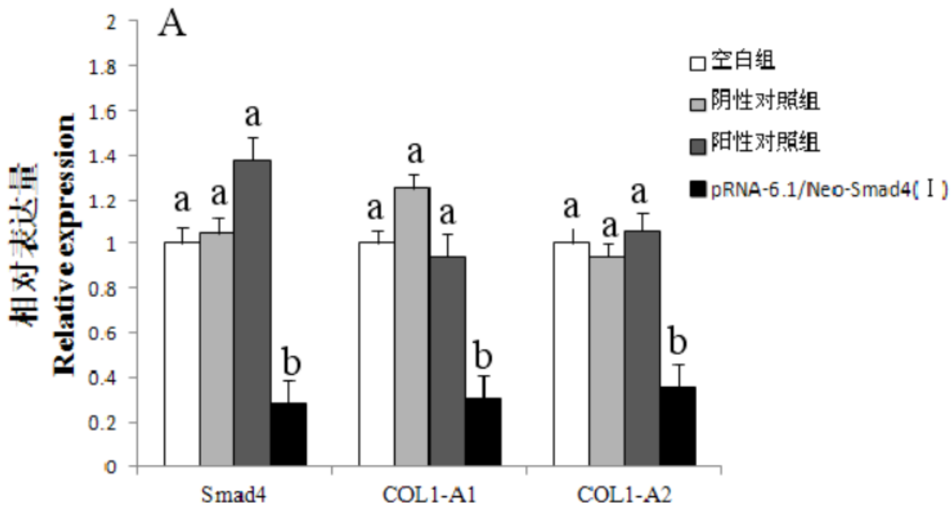


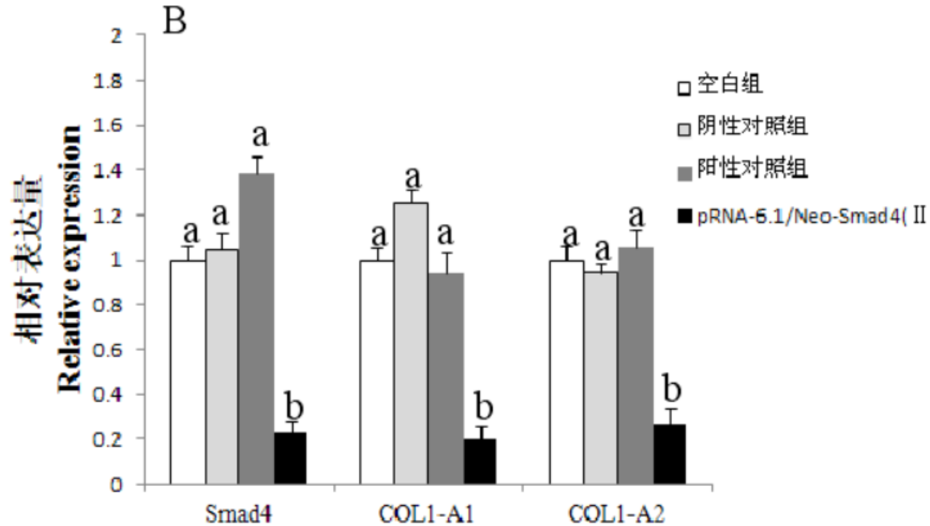


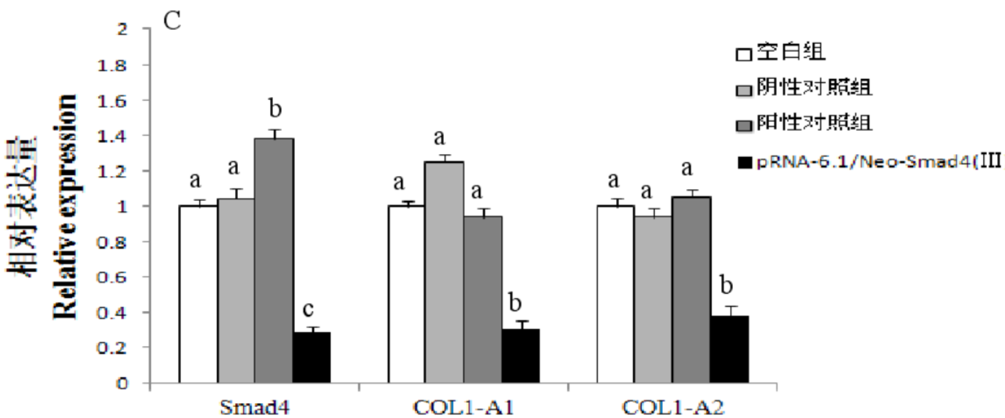
分别单独转染 pRNA-6.1/Neo-TGF-β1(I)、p RNA-6.1/Neo-TGF-β1(II)和 p RNA-6.1/Neo –TGF-β1(III) 干扰表达载体 36h 后 *TGF-β1, COL1-A1, COL1-A2* 相对表达量：A 为转染 pRNA-6.1/Neo-TGF-β1(I)干扰表达载体相对表达量， B 为转染 pRNA-6.1/Neo-TGF-β1(II)干扰表达载体相对表达量, C 为转染 p RNA-6.1/Neo-TGF-β1(III)干扰表达载体相对表达量， 不同字母代表相互之间存在显著性差异（$P<0.05$）

4 分析与讨论

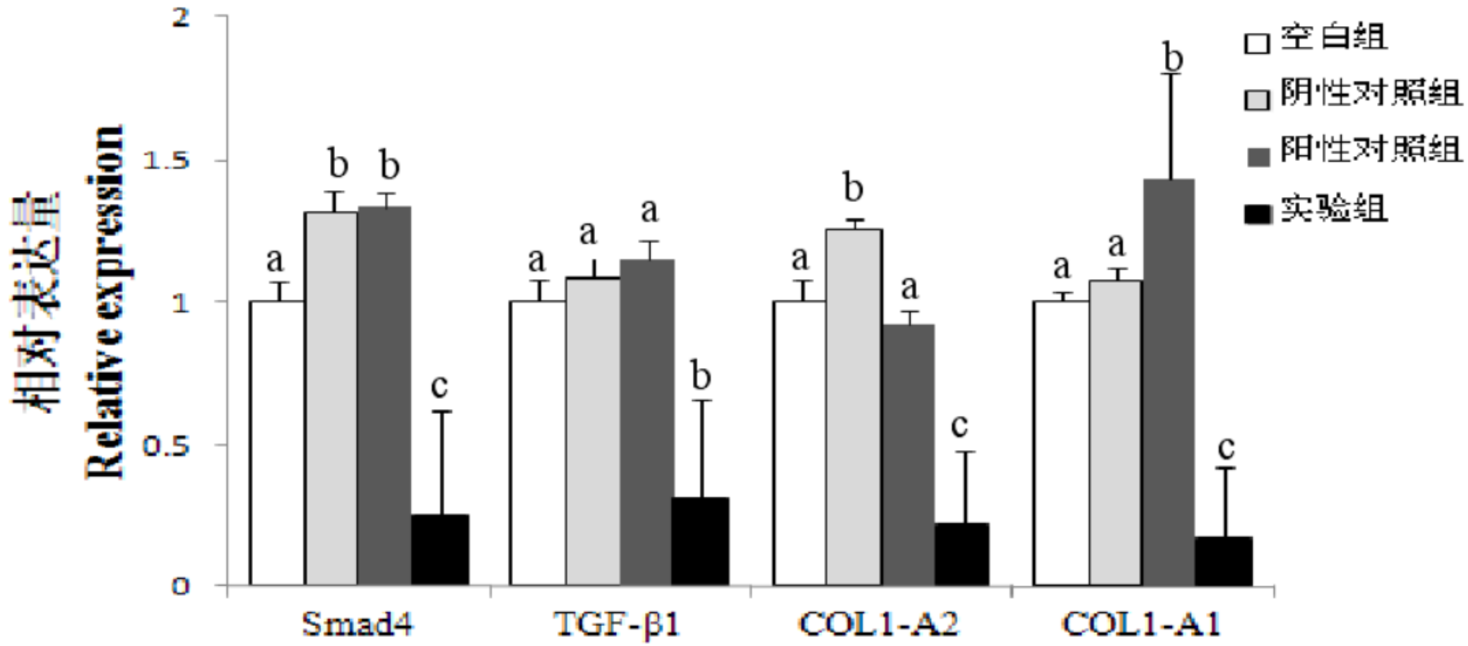
ANALYSIS AND DISCUSSION





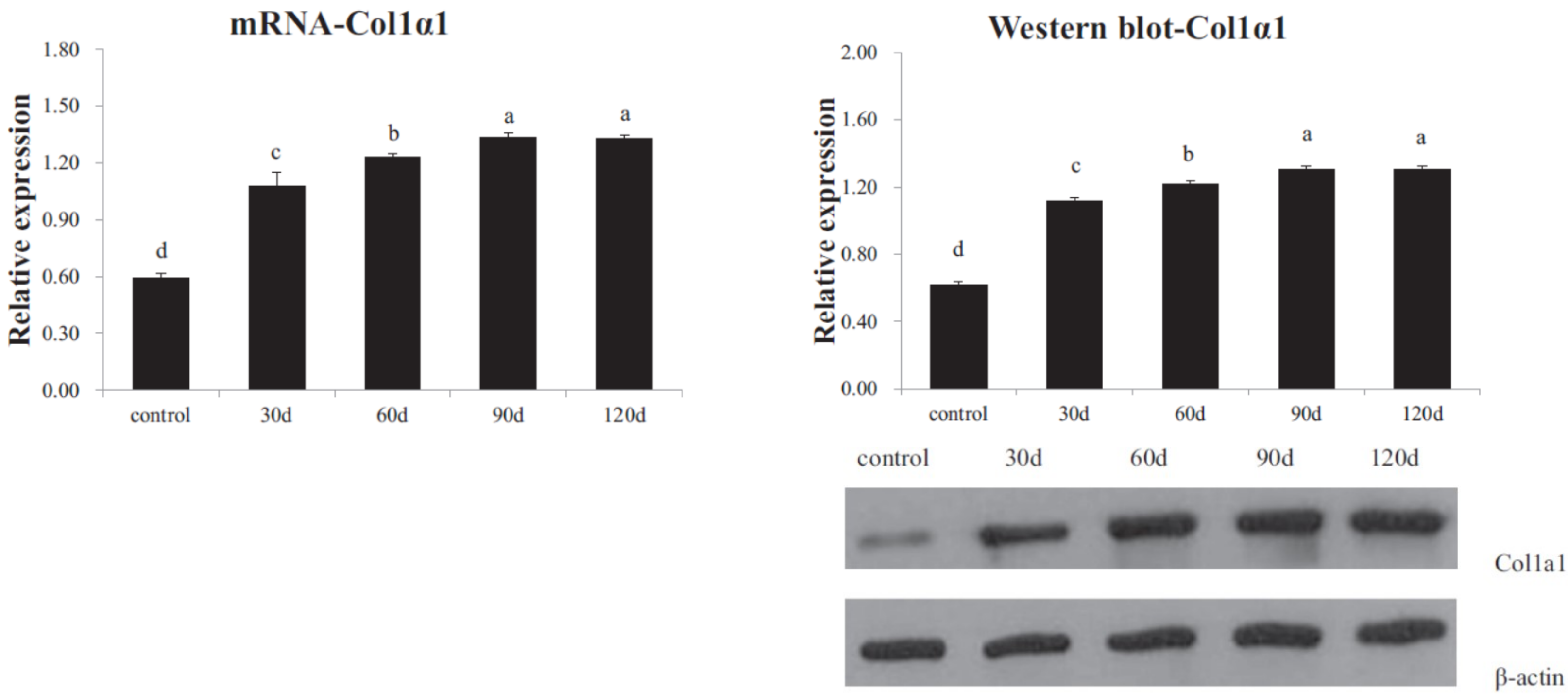


分别单独转染 p RNA-6.1/Neo-Smad4(I)、p RNA-6.1/Neo-Smad4(II)和 p RNA-6.1/Neo-Smad4(III) 干扰表达载体 36h 后 Sm*ad4*、*COL1-A1*、*COL1-A2* 相对表达量：A 为转染 pRNA-6.1/Neo-Smad4(I)干扰表达载体相对表达量，B 为转染 pRNA-6.1/Neo-Smad4(II)干扰表达载体相对表达量, C 为转染 pRNA-6.1/Neo -Smad4(III)干扰表达载体相对表达量，不同字母代表相互之间存在显著性差异（P<0.05）



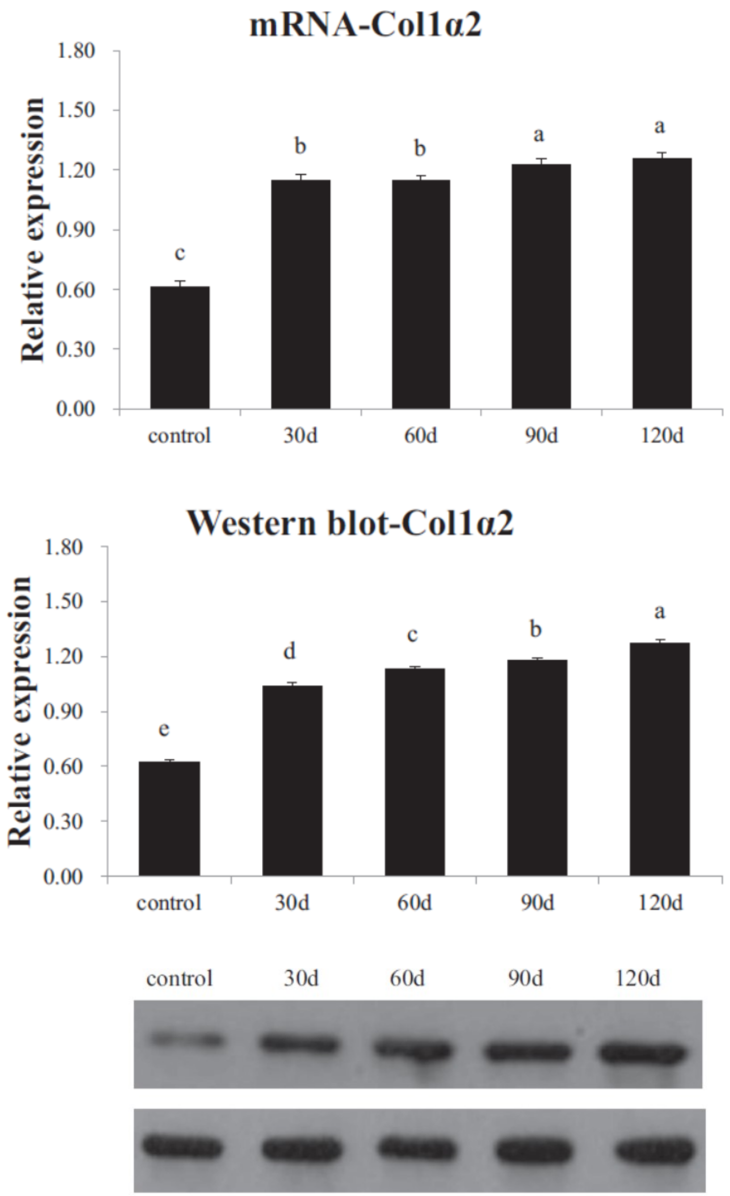
同时转染 pRNA-6.1/Neo-TGF-β1(III)和 p RNA-6.1/Neo-Smad4(II)干扰表达载体 36h 后*TGF-β1*、*Smad4*、*COL1-A1*、*COL1-A2* 相对表达量，不同字母代表相互之间存在显著性差异（$P<0.05$）

4 分析与讨论 ANALYSIS AND DISCUSSION



Relative mRNA and protein expressions of *Col1α1* in the muscle of grasscarp (crisp grass carp fed with faba bean/the control). Control is the muscle of grass carp before the initiation of faba bean feeding. Groups 30 d, 60 d, 90 d and 120 d are crisp grass carp fed with faba bean for 30, 60, 90 and 120 days, respectively.
The mRNA levels of Col1α1 gene were detected by real-time PCR and were normalized to the levels of EF1a (internal control). Col1α1 proteins were detected by Western blot, and relative protein expression levels were calculated by using the Quantity One software using β-actin protein as the reference. Results are expressed as means ± standard error (bars in the graph) from three fish (n=3). Bars with different letters indicate the significant difference ($P < 0.05$). Col1α1, Collagen type I alpha 1.

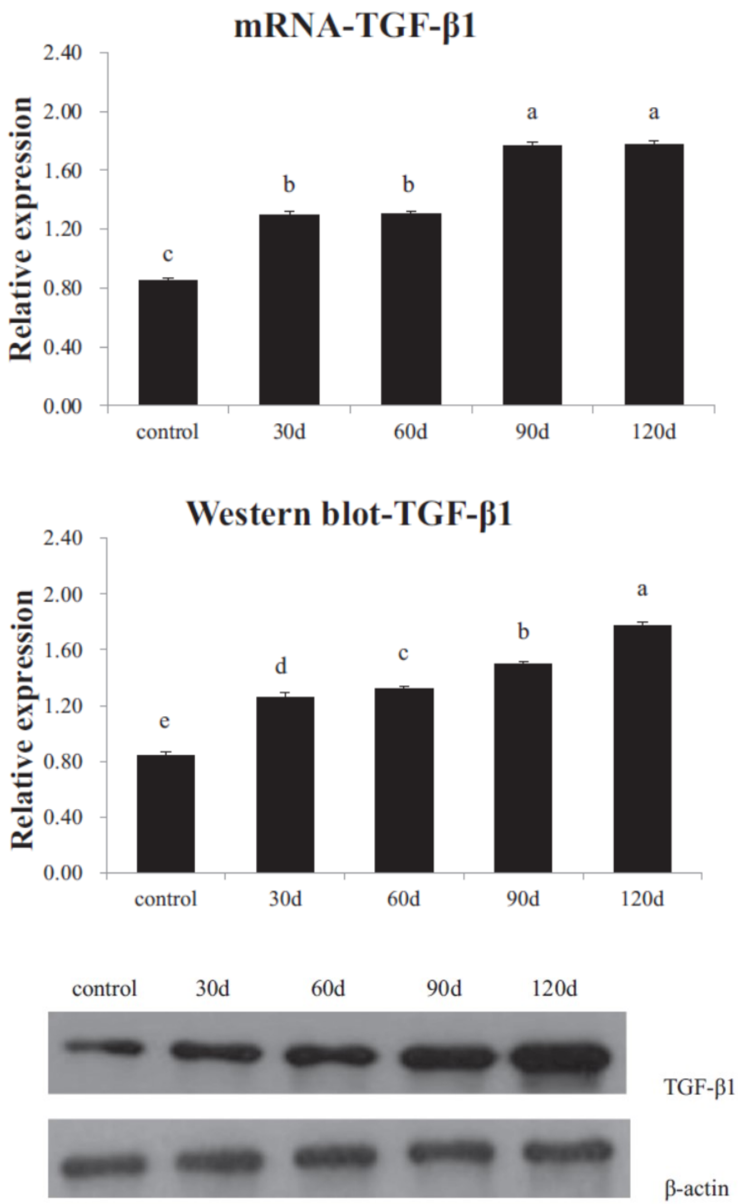
4 分析与讨论 ANALYSIS AND DISCUSSION



Relative mRNA and protein expressions of *Col1α2* in the muscle of grass carp (crisp grass carp fed with faba bean/the control). Control is the muscle of grass carp before the initiation of faba bean feeding. Groups 30 d, 60 d, 90 d and 120 d are crisp grass carp fed with faba bean for 30, 60, 90 and 120 days, respectively.

The levels of Col1α2 protein and mRNA were quantified by Western blot and real-time PCR, respectively, and relative protein expression levels was calculated as described Fig. 1 legend. Results are expressed as means ± S.E. (bars in the graph) from three fish (n=3). Bars with different letters indicate the significant difference ($P < 0.05$). Col1α2, Collagen type I alpha 2.

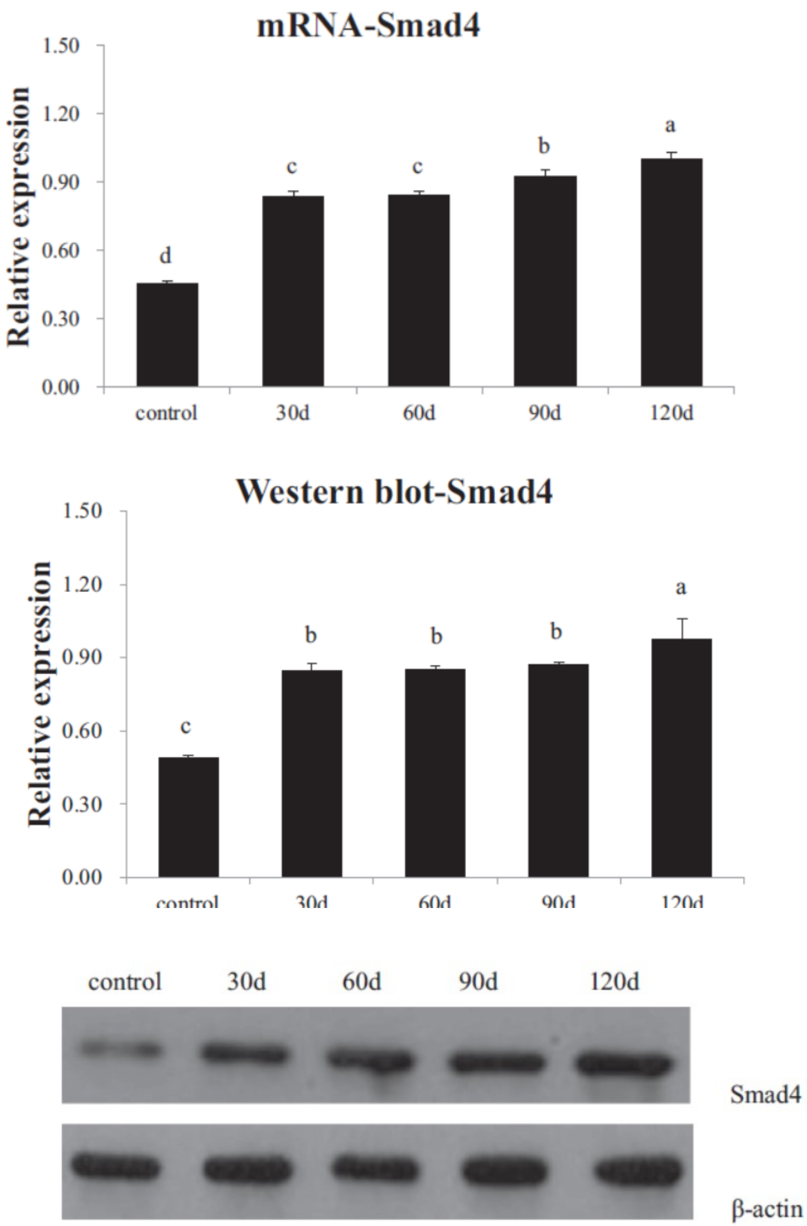
4 分析与讨论 ANALYSIS AND DISCUSSION



Relative mRNA and protein expressions of *TGF-β1* in the muscle of grass carp (crisp grass carp fed with faba bean/the control). Control is the muscle of grass carp before the initiation of faba bean feeding. Groups 30 d, 60 d, 90 d and 120 d are crisp grass carp fed with faba bean for 30, 60, 90 and 120 days, respectively.

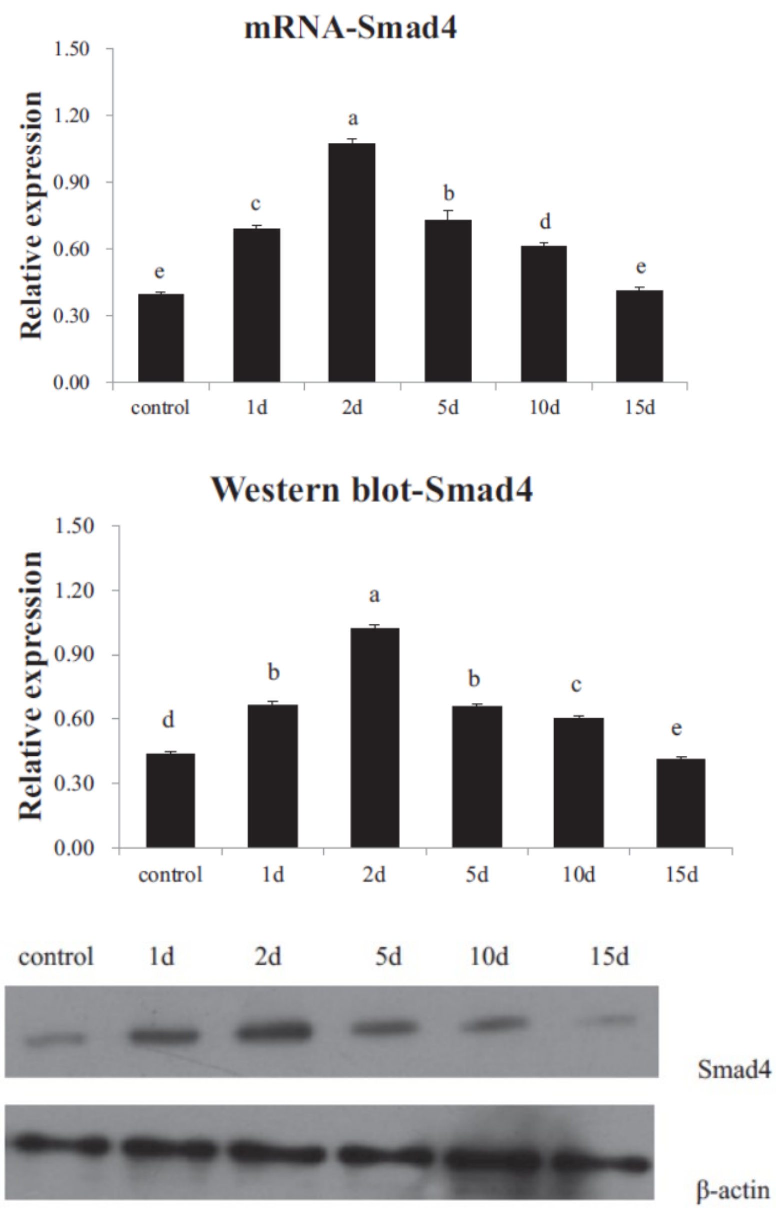
The levels of TGF-β1 protein and mRNA were quantified by Western blot and real-time PCR, respectively, and relative protein expression levels was calculated as described Figs.1 legend. Results are expressed as means±S.E. (bars in the graph) from three fish (n=3). Bars with different letters indicate the significant difference ($P < 0.05$).

4 分析与讨论 ANALYSIS AND DISCUSSION



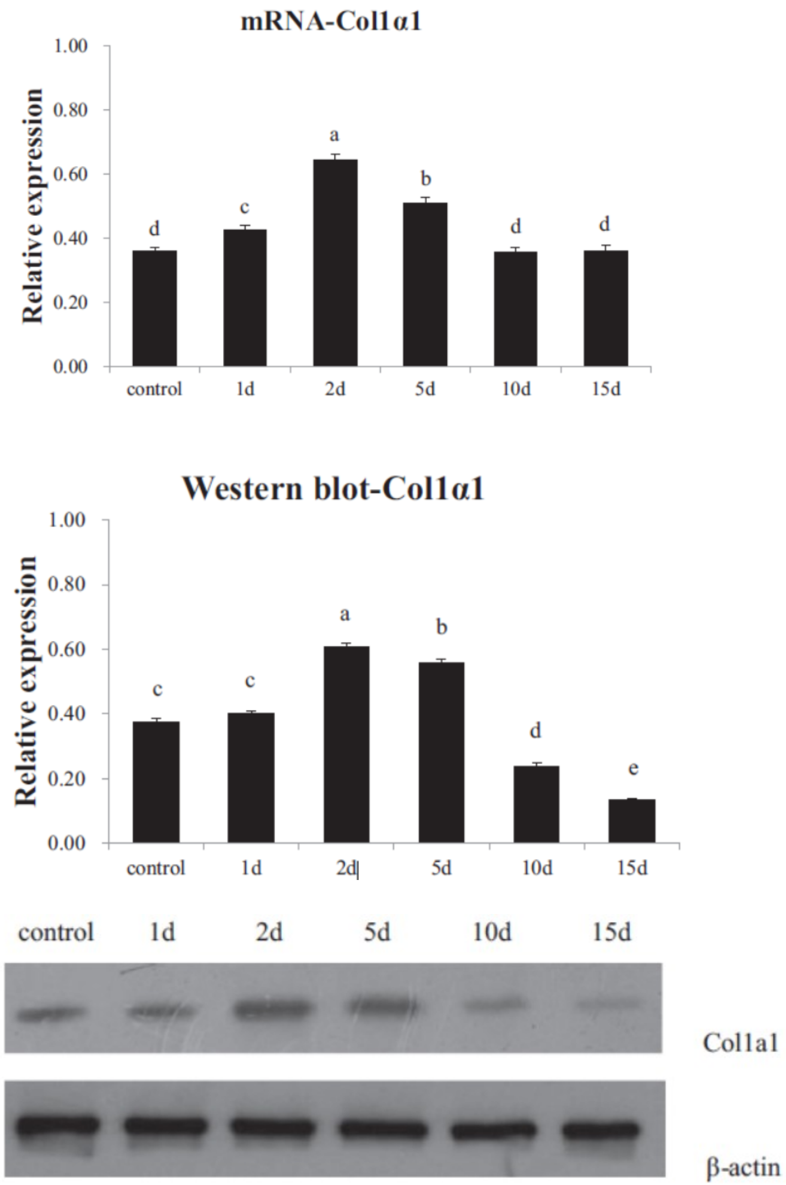
Relative mRNA and protein expressions of *Smad4* in the muscle of grass carp (crisp grass carp fed with faba bean/the control). Control is the muscle of grass carp before the initiation of faba bean feeding. Groups 30 d, 60 d, 90 d and 120 d are crisp grass carp fed with faba bean for 30, 60, 90 and 120 days, respectively. The levels of Smad4 protein and mRNA were quantified by Western blot and real-time PCR, respectively, and relative protein expression levels was calculated as described Figs.1 legend. Results are expressed as means±S.E. (bars in the graph) from three fish (n=3). Bars with different letters indicate the significant difference ($P < 0.05$).

4 分析与讨论 ANALYSIS AND DISCUSSION



Expressions of Smad4 in the muscle of crisp grass carp injected *Smad4* over-expression vector. Control fish were injected non-recombinant plasmid (pcDNA3.1(+)). Groups 1 d, 2 d, 5 d, 10 d,15 d are fish sampled 1, 2, 5, 10 and 15 days after injection, respectively. The levels of Smad4 protein and mRNA were quantified by Western blot and real-time PCR, respectively, and relative protein expression levels was calculated as described Figs.1 legend. Data are means±S.E. of three independent experiments, and different letters indicate significant differences ($P < 0.05$).

4 分析与讨论 ANALYSIS AND DISCUSSION

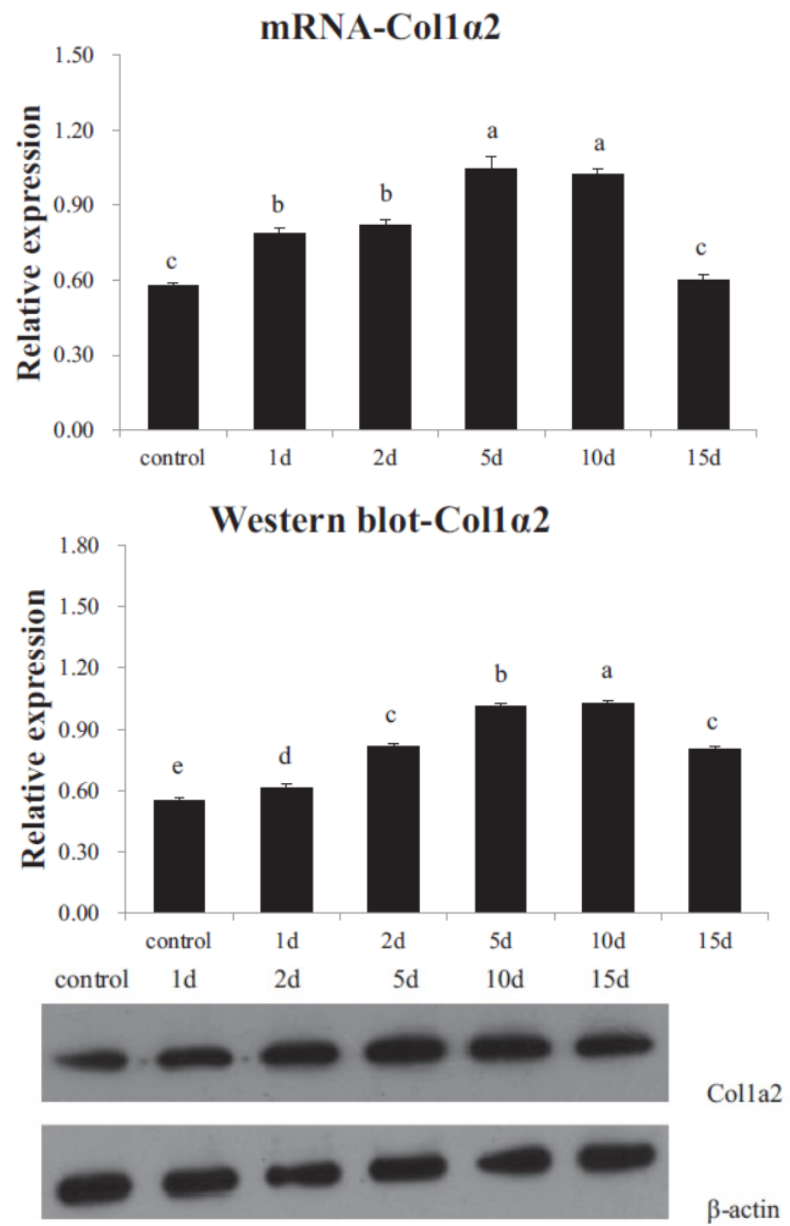


Expressions of Col1α1 in the muscle of crisp grass carp injected *Smad4* over-expression vector. Control fish were injected non-recombinant plasmid (pcDNA3.1(+)). Groups 1 d, 2 d, 5 d, 10 d,15 d are fish sampled 1, 2, 5, 10 and15 days after injection, respectively. The levels of Col1α1 protein and mRNA were quantified by Western blot and real-time PCR, respectively, and relative protein expression levels was calculated as described Figs.1 legend.

Data are means±S.E. of three independent experiments, and different letters indicate significant differences ($P < 0.05$).

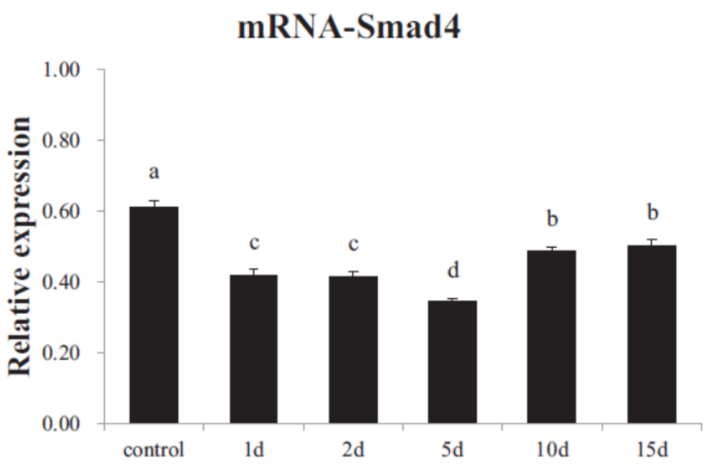
4 分析与讨论

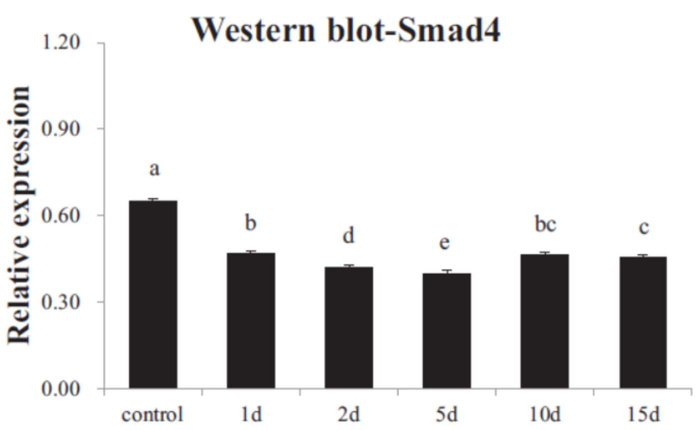
ANALYSIS AND DISCUSSION

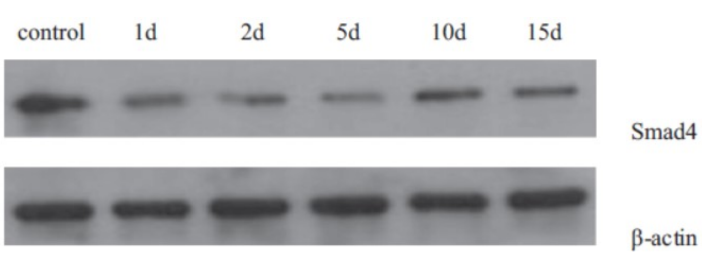


Expressions of Col1α2 in the muscle of crisp grass carp injected *Smad4* over-expression vector. Control fish were injected non-recombinant plasmid (pcDNA3.1(+)). Groups 1 d, 2 d, 5 d, 10 d,15 d are fish sampled 1, 2, 5, 10 and 15 days after injection, respectively. The levels of Col1α2 protein and mRNA were quantified by Western blot and real-time PCR, respectively, and relative protein expression levels was calculated as described Figs.1 legend.

Data are means±S.E. of three independent experiments, and different letters indicate significant differences ($P < 0.05$).

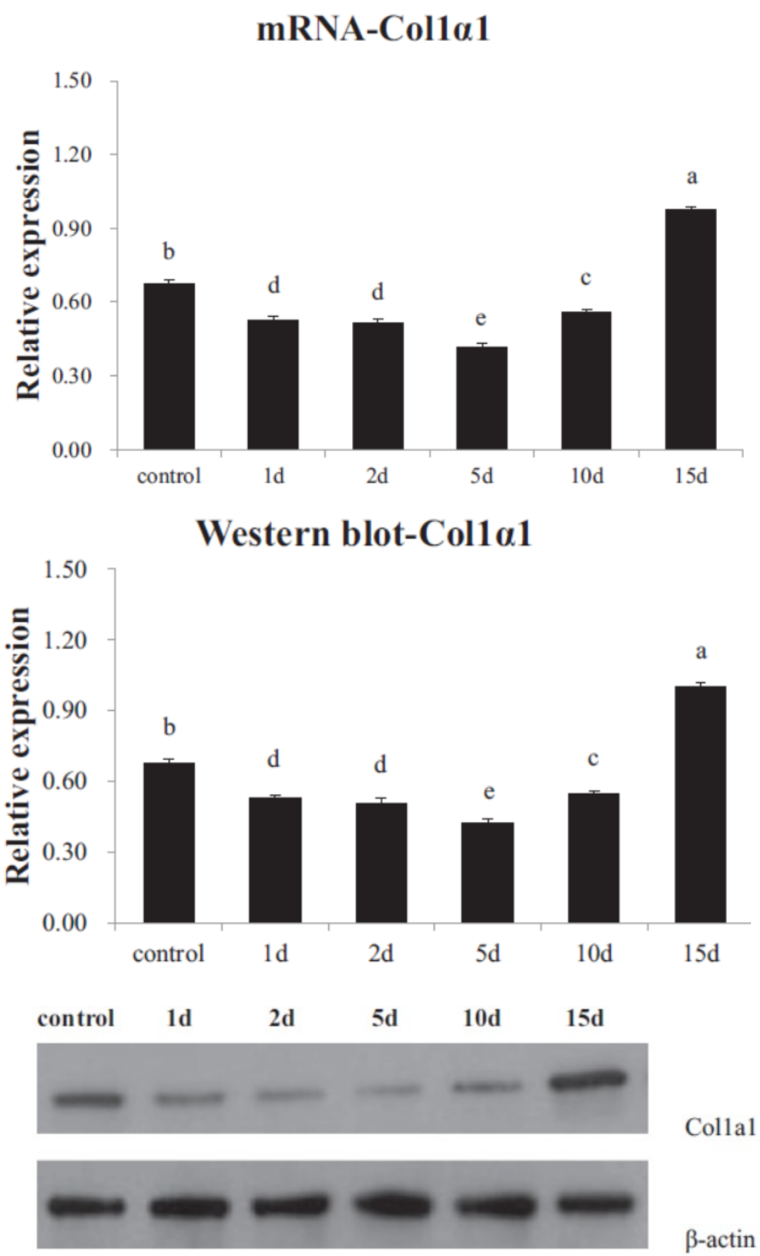






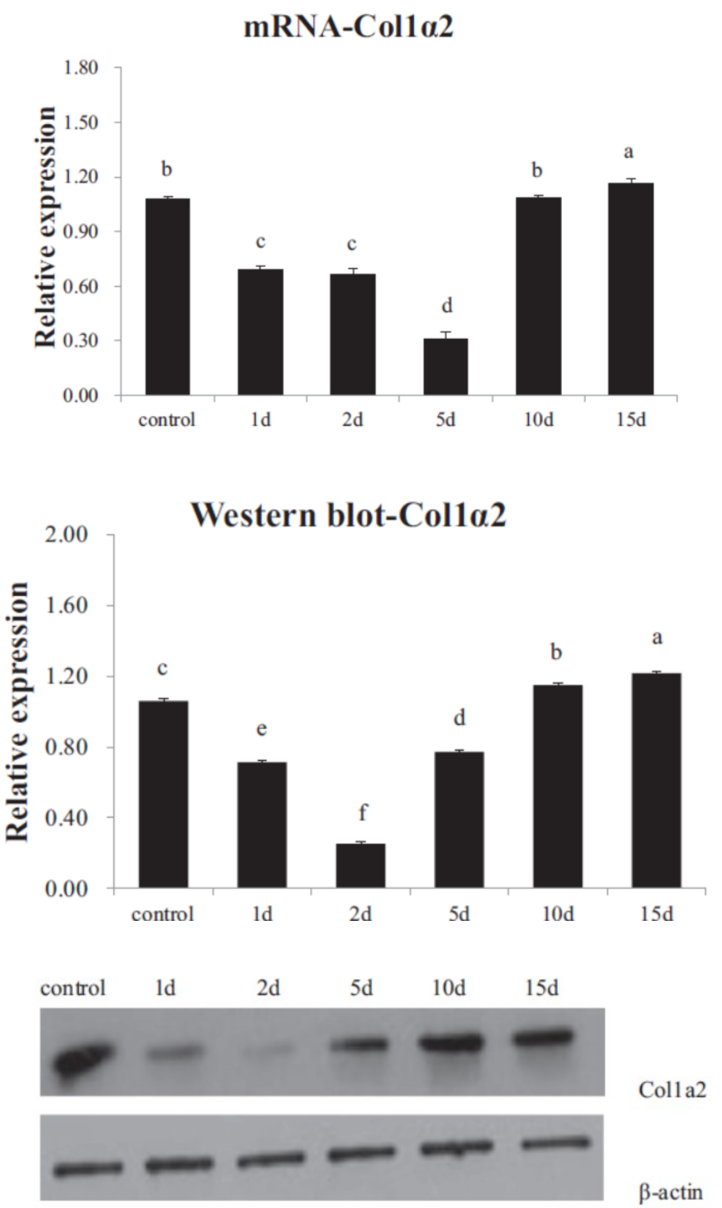
Expressions of Smad4 in the muscle of crisp grass carp injected *Smad4* RNAi vector. Control was injected using the non-recombinant plasmid (pRNAU6.1/ Neo). 1 d, 2 d, d, 10 d, 15 d were injected RNAi vector for 1 day, 2 days, 5 days,10 days and 15 days. The levels of Smad4 protein and mRNA were quantified by Western blot and real-time PCR, respectively, and relative protein expression levels was calculated as described Figs.1 legend. Data are means±S.E. of three independent experiments, and different letters indicated significant different ($P < 0.05$).

4 分析与讨论 ANALYSIS AND DISCUSSION



Expressions of *Col1α1* in the muscle of crisp grass carp injected Smad4 RNAi vector. Control was injected using the non-recombinant plasmid (pRNAU6.1/ Neo). 1 d, 2 d, 5 d, 1 d, 15 d were injected RNAi vector for 1 day, 2 days,5 days,10 days and 15 days. The levels of Col1α1 protein and mRNA were quantified by Western blot and real-time PCR, respectively, and relative protein expression levels was calculated as described Figs.1 legend. Data are means±S.E. of three independent experiments, and different letters indicatedsignificant different ($P < 0.05$).

4 分析与讨论 ANALYSIS AND DISCUSSION



Expressions of *Col1α2* in the muscle of crisp grass carp injected Smad4 RNAi vector. Control was injected using the non-recombinant plasmid (pRNAU6.1/ Neo). 1 d, 2 d, 5 d, 10 d,15 d were injected RNAi vector for 1 day, 2 days, 5 days,10 days and 15 days. The levels of Col1α2 protein and mRNA were quantified by Western blot and real-time PCR, respectively, and relative protein expression levels was calculated as described Fig. 1 legend. Data are means ± S.E. of three independent experiments, and different letters indicated significant different ($P < 0.05$).

5

CONCLUSION AND SUGGESTION

结论与建议

结论

In sum, this study demonstrated that in grass carp fed with faba bean, both mRNA and protein expressions of key factors of the TGF-β1/Smads pathway increase in association with the expression of type I collagen. Over-expression and knock down of Smad4 led to the significant increase and decrease of both mRNA and protein expressions of Col1α1 and Col1α2, respectively.

According to these results, Smad4 could be considered as an important regulator of type I collagen in crisp grass carp.

Our study will provide an important **mechanistic basis** for the regulation of type I collagen in the muscle of fish, and a reference for the nutritional regulation for **muscle hardness** of fish.

建议

- 格式上的问题比较多；
- 讨论部分没有对机制问题提出假设，谈论的文献多用的是哺乳动物的，应该增加从鱼类的其他物种的肌肉胶原蛋白含量与肉质的质构特性的相关性和草鱼基因组层面加以讨论；
- 柱状图比较粗糙

6

SUMMARY

总结

6 总结

SUMMARY

通过对郁二蒙、谢骏团队关于蚕豆对草鱼营养调控相关的文献整理和学习，对该领域的了解更深一步，进一步理解RNAi在细胞水平和个体水平调控的技术手段。

同时，也几个疑问：

- 蚕豆对鱼类的脆化，是否符合消费者对理想中淡水鱼类肉质质构的预期？
- 蚕豆对鱼类的脆化，是正常营养调控还是不正常的营养层面的环境胁迫？
- 技术层面，鲤科鱼类的基因组的复杂性，有没有更好的技术手段进行体内和体外的基因沉默或敲降？

THANKS