

## 黄芩素对猪前体脂肪细胞增殖分化的影响

# Effects of Baicalein on the Proliferation and Differentiation of Pig Preadipocyte

卢荣华 李 影 张立杰 杨公社\*

LU Rong-Hua, LI Ying, ZHANG Li-Jie and YANG Gong-She\*

西北农林科技大学动物脂肪沉积与肌肉发育实验室 杨凌 712100

Laboratory of Animal Fat Deposition and Muscle Development, Northwest A&F University, Yangling 712100, China

**摘 要** 研究黄芩素(BAI)对猪前体脂肪细胞增殖分化的影响,并探讨其可能的作用机制。原代培养猪前体脂肪细胞,采用油红 O 染色观察细胞分化的形态学变化;MTT 检测细胞增殖状况;油红 O 染色提取定量分析细胞内脂肪生成及细胞分化程度;分光光度法测定脂肪酸合酶(FAS)的活性;逆转录-聚合酶链反应(RT-PCR)检测分化特异基因过氧化物酶体增殖物激活受体 $\gamma$ (PPAR $\gamma$ ) mRNA 表达变化。结果显示,前体脂肪细胞在分化成脂肪细胞的过程中,其形态由梭形变成椭圆形、圆形,细胞内充满大小不一的脂滴;BAI 浓度在 160 ~ 640  $\mu$ mol/L 时显著抑制其增殖( $P < 0.05$ )。BAI 浓度为 40 ~ 320  $\mu$ mol/L 时显著抑制 PPAR $\gamma$  mRNA 表达和 FAS 的活性,并抑制细胞分化( $P < 0.05$ )。以上结果说明,BAI 对前体脂肪细胞增殖分化均有一定抑制作用,BAI 可能通过抑制 PPAR $\gamma$  mRNA 表达和降低 FAS 活性,从而抑制猪前体脂肪细胞分化。

**关键词** 黄芩素,猪,前体脂肪细胞,增殖,分化

中图分类号 Q253 文献标识码 A 文章编号 1000-306X(2006)06-1002-05

**Abstract** To investigate the effects of Baicalein (BAI) on the proliferation and differentiation of pig preadipocytes, and elucidate its potential mechanism. Primary preadipocytes of pig were cultured *in vitro*. The morphologic changes of preadipocytes differentiation were observed by Oil Red O staining. Status of cell proliferation was detected by MTT assay. The degree of adipogenesis and differentiation were measured by Oil Red O staining extraction assay. The activity of fatty acid synthase (FAS) was detected by spectrophotometry. The mRNA expression of special peroxisome proliferation activated receptor- $\gamma$  gene (PPAR $\gamma$ ) was detected by reverse transcriptase polymerase chain reaction (RT-PCR). When preadipocytes differentiated into adipocytes, the preadipocytes were changed from shuttle shape to oval or round, in which big and small lipid droplets were filled. The proliferation of preadipocytes was inhibited by the treatment of 160 ~ 640  $\mu$ mol/L BAI ( $P < 0.05$ ). The mRNA expression of PPAR $\gamma$  and FAS activity and the differentiation of preadipocytes was repressed by 40 ~ 320  $\mu$ mol/L BAI treatment ( $P < 0.05$ ). It is concluded that the proliferation and differentiation of preadipocytes is inhibited by BAI in some degree. The effect of BAI on differentiation of preadipocytes may be resulted from inhibiting the mRNA expression of PPAR $\gamma$  and reducing FAS activity.

**Key words** Baicalein, pig, preadipocyte, proliferation, differentiation

Received: May 12, 2006; Accepted: July 4, 2006.

This work was supported by the grants from the National Basic Research Program of China (973) (No. 2004CB117506) and the National Natural Science Foundation of China (No. 30471239).

\* Corresponding author. Tel 86-29-87091017; E-mail: gsyang999@hotmail.com

国家重点基础研究发展计划(973 计划)(No. 2004CB117506)和国家自然科学基金项目(No. 30471239)资助。

© 中国科学院微生物研究所期刊联合编辑部 <http://journals.im.ac.cn>

各浓度 BAI 处理猪前体脂肪细胞,MTT 法测定对细胞增殖的影响(见表 1)。由表 1 可见,与对照组相比,在细胞培养的 2、4、6、8 d,160、320、540  $\mu\text{mol/L}$  BAI 显著抑制前体脂肪细胞的增殖( $P <$

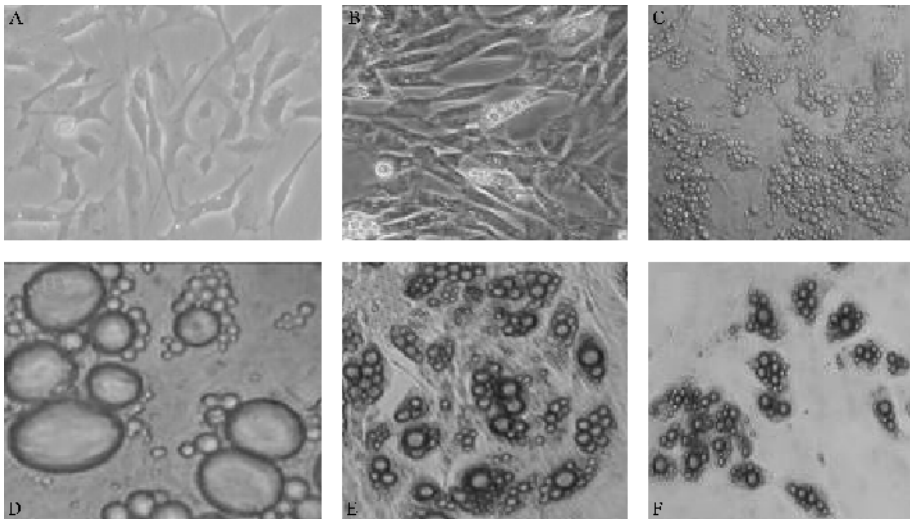


图 1 猪前体脂肪细胞分化的形态学变化及油红 O 染色

Fig. 1 Morphology change of pocine preadipocyte differentiation and Oil Red O staining

- A : 2 days culture , preadipocytes show undifferentiated and like the shuffle or irregular triangle (  $\times 200$  ) ;  
B : 4 days culture , some of preadipocytes began to differentiate into adipocytes and filled with small lipid droplets (  $\times 200$  ) ;  
C : 8 days culture , most of preadipocytes differentiated into adipocytes and be full of small lipid droplets (  $\times 100$  ) ;  
D : 10 days culture , preadipocytes differentiated into mature adipocytes and small lipid fused into big lipid droplets (  $\times 400$  ) ;  
E : Oil Red O staining on adipocytes ( control ) (  $\times 200$  ) ;  
F : Oil Red O staining on adipocytes treated with BAI , in which the amount of differentiated adipocytes was significantly less than control (  $\times 200$  ).

0.05 ) , 而  $40\mu\text{mol/L}$  BAI 处理组对细胞增殖的影响差异不显著 (  $P > 0.05$  ) 。 结果显示 ,BAI 浓度为  $160 \sim 640\mu\text{mol/L}$  时显著抑制猪前体脂肪细胞的增殖 (  $P < 0.05$  ) , 且随培养时间延长其抑制作用呈增强趋势。

表 1 MTT 比色结果  
Table 1 The result of MTT

Concentrations of BAI ( $\mu\text{mol/L}$ )	Culture time			
	2d	4d	6d	8d
Control	$0.101 \pm 0.002^a$	$0.166 \pm 0.005^a$	$0.310 \pm 0.009^a$	$0.305 \pm 0.003^a$
40	$0.097 \pm 0.004^a$	$0.163 \pm 0.008^a$	$0.295 \pm 0.010^a$	$0.292 \pm 0.011^a$
80	$0.096 \pm 0.004^a$	$0.162 \pm 0.003^a$	$0.257 \pm 0.006^b$	$0.250 \pm 0.008^b$
160	$0.084 \pm 0.002^b$	$0.139 \pm 0.002^b$	$0.220 \pm 0.004^c$	$0.210 \pm 0.002^c$
320	$0.074 \pm 0.001^c$	$0.121 \pm 0.002^c$	$0.186 \pm 0.008^d$	$0.176 \pm 0.003^d$
640	$0.065 \pm 0.003^d$	$0.106 \pm 0.005^d$	$0.141 \pm 0.005^e$	$0.127 \pm 0.004^e$

Different letters show significant differences (  $P < 0.05$  ) , same letters show no differences within the same time (  $P > 0.05$  ) (  $n = 3$  ).

2.3 BAI 对前体脂肪细胞分化的影响

由表 2 分析可知 ,BAI 短时间处理 ( 36h ) 后 ,除  $20\mu\text{mol/L}$  浓度与对照组没有显著差异外 (  $P > 0.05$  ) ,其余各浓度 BAI 均显著抑制前体脂肪细胞分化 (  $P < 0.05$  ) ;在 BAI 长时间处理 ( 72h ) 后 ,除  $20\mu\text{mol/L}$  浓度与对照组差异仍不显著外 (  $P > 0.05$  ) ,其余各处理组均显著抑制前体脂肪细胞分化 (  $P < 0.05$  ) ,且不同浓度 BAI 处理组之间也呈显著性差异 (  $P < 0.05$  ) ,依浓度增大抑制作用明显提高。可见  $40 \sim 320\mu\text{mol/L}$  BAI 可显著降低脂肪细胞内脂肪的生成量 ,对猪前体脂肪细胞向成熟脂肪细胞分化具有较强的抑制作用。

表 2 油红 O 比色结果  
Table 2 The result of Oil Red O

Concentrations of BAI ( $\mu\text{mol/L}$ )	Culture time	
	36h	72h
Control	$0.219 \pm 0.002^a$	$0.260 \pm 0.001^a$
20	$0.214 \pm 0.003^a$	$0.255 \pm 0.003^a$
40	$0.196 \pm 0.005^b$	$0.240 \pm 0.004^b$
80	$0.189 \pm 0.007^b$	$0.191 \pm 0.005^c$
160	$0.124 \pm 0.003^c$	$0.131 \pm 0.002^d$
320	$0.083 \pm 0.003^d$	$0.088 \pm 0.002^e$

Different letters show significant differences (  $P < 0.05$  ) , same letters show no differences within the same time (  $P > 0.05$  ) (  $n = 3$  ).

2.4 BAI 对脂肪细胞 PPAR $\gamma$ 2 mRNA 表达的影响  
分别以对照、 $40\mu\text{mol/L}$ 、 $160\mu\text{mol/L}$  BAI 作用于成熟脂肪细胞 48h , PPAR $\gamma$ 2 总 RNA 经 RT-PCR 后的产

物进行电泳 ,Doliphin-DOC 凝胶成像仪成像(图 2-A),成像系统软件测定各条带的 A 值 ,经内标  $\beta$ -actin mRNA 校正 ,结果见图 2-B。经 Dolphin-DOC 凝胶成像系统软件分析 ,40、160 $\mu$ mol/L 组与对照组比较 PPAR $\gamma$ 2 mRNA 表达量显著降低(  $P < 0.01$  ),且 160 $\mu$ mol/L 组与 40 $\mu$ mol/L 组比较差异显著(  $P < 0.05$  )。表明 BAI 处理后 ,呈剂量依赖性的抑制 PPAR $\gamma$ 2 mRNA 表达。

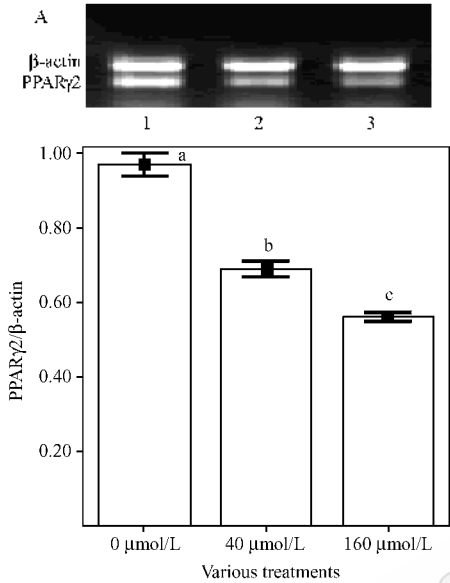


图2 PPAR $\gamma$ 2 及内参  $\beta$ -actin 基因 RT-PCR 产物电泳图(A)及各组脂肪细胞 PPAR $\gamma$ 2 mRNA 的表达(B)

Fig.2 Electrophoresis result of PPAR $\gamma$ 2 and  $\beta$ -actin RT-PCR products(A), and mRNA expression of PPAR $\gamma$ 2 in adipocytes (B)

A : 1. control ; 2. 40 $\mu$ mol/L BAI treated ; 3. 160 $\mu$ mol/L BAI treated. B : different letters denote significant difference (  $P < 0.05$  )(  $n = 3$  ).

2.5 BAI 对 FAS 活性的影响

在加入不同浓度 BAI 后 FAS 的活性见图 3。由图可知 ,和对照组相比 ,随处理浓度的升高 ,FAS 的活性显著降低(  $P < 0.05$  ),而且 160 $\mu$ mol/L 组与 40 $\mu$ mol/L 组比较差异显著(  $P < 0.05$  )。表明 BAI 对脂肪细胞分化的抑制作用与 FAS 的活性降低有关 ,且 BAI 成剂量依赖性抑制 FAS 的活性。

3 讨论

3.1 BAI 对猪前体脂肪细胞增殖的影响

MTT 法检测结果表明 ,在整个培养过程中高浓度 BAI( 160 ~ 640 $\mu$ mol/L )显著抑制前体脂肪细胞的增殖。已有研究显示 ,BAI 对多种细胞如血管内皮细胞、人胃癌细胞、大鼠肝星状细胞等<sup>[2,11]</sup>具抗增殖

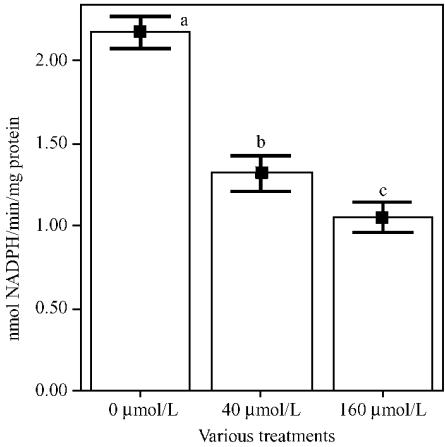


图3 BAI 对 FAS 活性的影响

Fig.3 The effects of BAI on FAS activity  
Different letters denote significant difference  
(  $P < 0.05$  )(  $n = 3$  ).

的作用 ,而其作用机理大多通过诱导细胞凋亡而发挥作用。高浓度的 BAI 对猪前体脂肪细胞的增殖具抑制作用 ,这有助于降低体脂沉积。其抑制机理可能是高浓度的 BAI 对细胞的生长也具有诱导凋亡作用 ,但具体的机制尚需进一步证明。

3.2 BAI 对猪前体脂肪细胞分化的影响

本研究发现 ,与对照组相比 ,40 ~ 320 $\mu$ mol/L 浓度的 BAI 均抑制猪前体脂肪细胞的分化 ,并抑制 FAS 的活性 ,而这可能是 BAI 影响其分化的机制之一 ,因为脂肪合成是前体脂肪细胞分化为脂肪细胞过程中重要的生化反应。Loftus 等<sup>[12]</sup>报道 ,用 FAS 抑制剂注射使肥胖小鼠大幅度降低食欲和体重 ,提出 FAS 与食欲调控之间可能存在重要联系 ,是治疗肥胖症的潜在靶部位 ,通过调控 FAS 的表达可以用来控制体脂沉积。成俊英等<sup>[3]</sup>研究也发现 ,BAI 能剂量依赖性抑制 3T3-L1 小鼠前体脂肪细胞分化和 FAS 活性。但与本实验所作用浓度明显不同 ,这可能是细胞来源不同所致。前体脂肪细胞的分化是一系列脂肪细胞分化标志基因顺次表达的过程<sup>[13]</sup> ,主要受一系列转录因子的调控 ,其中 PPAR $\gamma$  在前体脂肪细胞的分化过程中具有重要调控作用。研究表明 PPAR $\gamma$  是脂肪细胞分化必需的<sup>[14]</sup> ,PPAR $\gamma$  被其配体激活后与 RXR 形成异二聚体 ,结合于过氧化物酶体增殖物反应元件( PPRE ) ,启动基因的表达 ,诱导脂肪细胞的分化 ,尽管许多因子参与脂肪细胞的分化 ,但都通过 PPAR $\gamma$  起作用 ,体外的 PPAR $\gamma$  的配体或激活物也均能诱导前体脂肪细胞分化<sup>[15]</sup>。但 BAI 调控 PPAR $\gamma$  基因的表达目前尚未见报道。杂合的

PPAR $\gamma$  基因缺失小鼠在高脂饮食情况下不会发生肥胖和胰岛素抵抗,说明降低 PPAR $\alpha$  表达能防止高脂饮食引起的脂肪细胞过度肥大和胰岛素抵抗<sup>[16]</sup>。本研究发现 BAI 抑制猪前体脂肪细胞分化可能还通过抑制 PPAR $\gamma$ 2 基因的表达来实现。提示 BAI 对于肥胖症的治疗具有重要的意义。

中药降脂的机制研究尚不够深入,其降脂作用较复杂,通常是通过多成分、多途径而起综合作用,BAI 抑制猪前体脂肪细胞的分化作用正好说明了这一点。中药降脂以其确切的疗效和安全、低毒等优势,具有广阔的开发前景。由于本研究是 BAI 抑制猪前体脂肪细胞增殖分化作用的初步探讨,其确切机制尚有待于进一步研究。

## REFERENCES(参考文献)

- [ 1 ] Wickelgren I. Obesity : how big a problem? *Science*, 1998, **280** ( 5368 ): 1364 - 1367
- [ 2 ] Huang CY( 黄彩云 ), Chen FL( 陈丰霖 ), Li JY( 李建英 ) *et al.* Expression of 12-lipoxygenase and its inhibitor's effect on proliferation of human AGS cell line. *World Chinese Journal of Digestology*( 世界华人消化杂志 ), 2005, **13**( 14 ): 1652 - 1657
- [ 3 ] Cheng JY( 成俊英 ), Zhang XL( 张晓岚 ), Yang Y( 杨扬 ) *et al.* Effects of baicalein on differentiation of 3T3-L1 preadipocytes into adipocytes and on fatty acid synthase activity. *Natural Product Research and Development*( 天然产物研究与开发 ) 2004, **16**( 1 ): 7 - 9
- [ 4 ] Dong QH( 董庆华 ), Zheng SC( 郑树 ), Xu RZ( 徐荣臻 ) *et al.* Baicalein selectively induce apoptosis in human leukemia K562 cells. *Acta Pharmaceutica Sinica*( 药学报 ) 2003, **38**( 11 ): 817 - 820
- [ 5 ] Rapacz J, Hasler RJ. Animal models : the pig genetic factors in atherosclerosis. *Approaches and Model Systems*, 1989, **12**: 139 - 169
- [ 6 ] Green E. Comparative sequencing of targeted regions in multiple vertebrates : Reconnaissance for future genome explorations. Plenary Lecture at Plant, Animal & Microbial Genome meeting, San Diego, Ca, 2002
- [ 7 ] Li Y( 李影 ), Yang GS( 杨公社 ), Lu RH( 卢荣华 ) *et al.* Optimal culture method of the porcine preadipocyte. *Chinese Journal of Cell Biology*( 细胞生物学杂志 ) 2005, **27**( 6 ): 697 - 700
- [ 8 ] Situ ZQ( 司徒镇强 ), Wu JZ( 吴军正 ). *Cell Culture*. Xi'an : World Publishing Corp 2004, pp. 250 - 252
- [ 9 ] Ramirez JL, Munozledo FC, Harcuch W. Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with Oil Red O. *Histochemistry*, 1992, **97**( 6 ): 493 - 497
- [ 10 ] Naima M, Brynn HJ, James WT. Insulin increases lipogenic enzyme activity in human adipocytes in primary culture. *The Journal of Nutrition*, 1996, **126**( 4 ): 865 - 870
- [ 11 ] Inoue T, Jackson E. Strong antiproliferative effects of baicalein in cultured rat hepatic stellate cells. *European Journal of Pharmacology*, 1999, **378**( 1 ): 129 - 135
- [ 12 ] Loftus TM, Jaworsky DE, Frehywot GL *et al.* Reduced food intake and body weight in mice treated with fatty acid synthase inhibition. *Science*, 2000, **288**( 30 ): 3450 - 3454
- [ 13 ] Fajas L, Fruchart JC, Auwerx J. Transcriptional control of adipogenesis. *Cell Biology*, 1998, **10**( 2 ): 165 - 173
- [ 14 ] Rosen ED, Sarraf P, Troy AE *et al.* PPAR $\gamma$  is required for the differentiation of adipose tissue *in vivo* and *in vitro*. *Mol Cell*, 1999, **4**: 611 - 617
- [ 15 ] Rosen ED, Hus CH, Wang X *et al.* C/EBP  $\alpha$  induces adipogenesis through PPAR  $\gamma$ : a unified pathway. *Genes Dev*, 2002, **16**: 22 - 26
- [ 16 ] Kadowaki T, Hara K, Kubota N *et al.* The role of PPAR $\gamma$  in high-fat diet-induced obesity and insulin resistance. *J Diabetes Complications*, 2002, **16**( 1 ): 41 - 45