分类号：　　密　　级：

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石河子大学

硕 士 学 位 论 文

ADRB3 激活促进棕色脂肪细胞 IL-6 表达的作用及机制研究

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申请学位门类级别　医学硕士

学科、专业名称　免疫学

研 究 方 向　肥胖及其相关代谢性疾病

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ADRB3 activation promotes IL-6 expression in brown adipocytes

A Dissertation Submitted to

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In Partial Fulfillment of the Requirements

for the Degree of

Master of Medical

By Liang mao-di

(Immunology)

Dissertation Supervisor: Prof. Zhang Jun. Xie Jian-xin. Wang Cui-zhe

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摘 要

目的：

最近的研究表明，急性应激状态下β3-肾上腺素受体(β3-adrenoceptor，ADRB3)激活，可通过促进棕色脂肪细胞 IL-6 的产生和释放增强肝脏糖异生，为机体应对应激提供能量。然而，ADRB3 激活后促进 IL-6 产生和释放的具体分子机制尚未见文献报道。ADRB3 激活后通过 cAMP-PKA-CREB 途径在脂肪组织产热、脂肪分解和葡萄糖摄取等过程中发挥重要作用。前期研究发现：转录因子 KLF7 可靶向上调脂肪细胞 IL-6 的表达。生物信息学预测提示：*KLF7* 启动子区存在多个 CREB 的结合位点。本研究在构建急性应激、ADRB3 激活小鼠模型，以及体外培养棕色脂肪细胞和小鼠肝细胞的基础上，明确急性应激状态下 ADRB3 激活后是否通过 cAMP-PKA-CREB-KLF7 信号通路上调棕色脂肪细胞 IL-6 的表达，将为阐明和丰富棕色脂肪细胞在急性应激状态下的全新功能提供新的理论依据。

方法：

1. 体内动物实验分组及检测指标

(1) 实验动物分组：40 只 6 周龄 C57BL/6 雄性小鼠，分为对照组(n =10)、急性应激组(n =10)、急性应激+ADRB3 拮抗剂组(n = 10)和急性应激+PKA 抑制剂组(n = 10)；40 只 6 周龄 C57BL/6 雄性小鼠，分为对照组(n =10)、ADRB3 激动剂(n =10)、ADRB3 激动剂+ADRB3 拮抗剂组(n = 10)和 ADRB3 激动剂+PKA 抑制剂组(n = 10)。

(2) 实验动物处理方式：急性应激组使用毛细吸管穿刺小鼠眼眶后静脉丛，对照组不做处理；ADRB3 激动剂处理组腹腔注射 1 mg/kg CL316,243 处理 2 h，对照组腹腔注射等量生理盐水；ADRB3 拮抗剂组小鼠腹腔注射 10 mg/kg SR59203A 预处理 1 h，对照组腹腔注射等量溶剂(生理盐水+5% DMSO)；PKA 抑制剂组给予小鼠腹腔注射 5 mg/kg H89 预处理 1 h，对照组给予等量溶剂(生理盐水+5% DMSO)。

(3) 检测指标和方法：Elisa 法检测血清中 IL-6 含量；qRT-PCR 和 Western Blot 检测棕色脂肪组织 p-PKA substrate、p-CREB、KLF7、IL-6 及肝脏糖异生相关基因表达水平；葡萄糖耐量试验(Glucose Tolerance Test, GTT)及胰岛素耐受试验(Insulin Tolerance Test, ITT)评价小鼠葡萄糖耐量及胰岛素敏感性；丙酮酸耐量(Pyruvate tolerance test, PTT)评价小鼠糖异生能力。

2.棕色脂肪细胞体外培养、处理及检测

(1) 体外原代培养小鼠棕色脂肪细胞、体外培养小鼠成纤维细胞系 C3H10T1/2，加入诱导剂诱导分化为成熟棕色脂肪细胞后，使用油红 O 染色法鉴定细胞中的脂滴，qRT-PCR 和 Western Blot 鉴定棕色脂肪标志物。

(2) 对原代培养以及 C3H10T1/2 诱导的成熟棕色脂肪细胞进行如下处理：ADRB3 激动剂处理组

使用 5 μM CL316,243 处理 4 h，对照组使用等量的生理盐水；腺苷酸环化酶激活剂处理组使用 10 μM Forskolin 处理 4 h，对照组使用等量的 DMSO 溶液；PKA 抑制剂处理组使用 10 μM H89 预处理 4 h，对照组使用等量的 DMSO 溶液。

收集 CL316,243 或 Forskolin 处理的棕色脂肪细胞的上清液，分别加入 2 μg/mL 的 IL-6 中和抗体或同型对照，与小鼠肝细胞 Hepa 1-6 共培养 4 h 后收集样本。

(3) 检测指标及方法：qRT-PCR 检测棕色脂肪细胞中 KLF7、IL-6，以及小鼠肝细胞 Hepa 1-6 中 *Pck1*、*Gck* 等糖异生相关基因的 mRNA 表达水平；Western Blot 检测 p-PKA substrate、p-CREB、KLF7 和 IL-6 的蛋白表达水平；Elisa 试剂盒检测细胞上清液中 IL-6 的含量。

3.生物信息学预测及验证

运用转录因子结合位点信息数据库预测转录因子 CREB 与 *KLF7* 启动子区的结合位点。体外培养 HEK-293T 细胞，运用双荧光素酶报告基因实验和染色质免疫共沉淀实验(Chromatin Immunoprecipitation，ChIP)验证 CREB 对 *KLF7* 的靶向调控作用。

4.统计学方法

运用 SPSS 26.0 进行数据分析。首先进行正态性检验，符合正态分布的两组数据使用 *t* 检验，不符合正态分布的两组数据使用秩和检验；符合正态分布的多组数据采用单因素方差分析。$P<0.05$ 认为差异有统计学意义。

结果：

1. 急性应激状态下 ADRB3 激活可上调棕色脂肪细胞 KLF7 和 IL-6 的表达

1.1、ADRB3 激活后可上调小鼠棕色脂肪组织 KLF7 和 IL-6 的表达，促进肝脏糖异生

运用两种方法构建 ADRB3 激活的小鼠模型：一种是眶后出血法构建急性应激小鼠模型激活 ADRB3；另一种是给予小鼠腹腔注射 ADRB3 激动剂 CL316,243 直接激活 ADRB3，分别进行如下实验：

与对照组相比，眶后出血应激小鼠模型棕色脂肪组织中 ADRB3 下游信号分子 p-PKA substrate、p-CREB、KLF7、IL-6 的表达水平显著增加；小鼠血清 IL-6 含量、血糖水平显著升高，GTT 和 ITT 无显著变化；急性应激小鼠肝脏组织中 *Pck1*、*Gck* 等糖异生相关基因的 mRNA 表达水平增加，糖异生能力显著增强。腹腔注射 ADRB3 拮抗剂 SR59203A 后，应激小鼠的上述表型得到了显著逆转。以上差异均具有统计学意义($P<0.05$)。在腹腔注射 ADRB3 激动剂 CL316,243 直接激活 ADRB3 的小鼠模型中得到与上述一致的结果。

1.2、原代脂肪细胞和 C3H10T1/2 小鼠前脂肪细胞的诱导分化

处死 2-4 周龄小鼠，分离肩胛区棕色脂肪组织中原代脂肪细胞，以及体外培养小鼠 C3H10T1/2 前脂肪细胞，诱导分化为成熟的棕色脂肪细胞。经油红 O 染色，镜下观察发现大量红染的小脂滴聚集；*Ucp1*、*Pgc1-α*、*Ppar-α*等产热相关基因的 mRNA 表达水平显著增加，表明棕色脂肪细胞诱导成功。以上差异具有统计学意义($P<0.05$)。

1.3、ADRB3 激活可显著促进棕色脂肪细胞 KLF7 和 IL-6 的表达

使用 ADRB3 激动剂 CL316,243 处理原代培养的小鼠棕色脂肪细胞 4 h 后，与对照组相比，棕色脂肪细胞中 ADRB3 下游信号分子 p-PKA substrate、p-CREB、KLF7、IL-6 的表达水平显著增加，细胞上清 IL-6 含量显著升高。收集棕色脂肪细胞上清与小鼠肝细胞 Hepa 1-6 共培养 4 h 后，CL316,243 处理的脂肪细胞上清可显著促进肝细胞糖异生相关基因 *Pck1*、*Gck*、*G6pc* 的表达，而使用 2 μg/mL IL-6 中和抗体显著逆转了 CL316,243 的上述作用。以上差异具有统计学意义(P<0.05)。在 C3H10T1/2 棕色脂肪细胞中得到与上述一致的结果。

2. ADRB3 激活后通过 KLF7 促进棕色脂肪细胞中 IL-6 的表达

与对照组相比，干扰 KLF7 后原代棕色脂肪细胞中 IL-6 的表达水平显著降低，细胞上清中 IL-6 含量显著下降；与 NC 组相比，过表达 KLF7 可显著促进原代棕色脂肪细胞中 IL-6 的表达水平，以及细胞上清中 IL-6 含量；与单独 CL316,243 处理组相比，CL316,243 处理细胞的同时，下调 KLF7 表达可显著逆转 ADRB3 激动剂对原代棕色脂肪细胞中 IL-6 表达的上调作用。以上差异具有统计学意义(P<0.05)。

3. ADRB3 激活可通过 cAMP-PKA-CREB 信号通路上调棕色脂肪细胞 KLF7 和 IL-6 表达

3.1、PKA 抑制剂可逆转急性应激对棕色脂肪组织 KLF7 和 IL-6 表达的上调作用

与对照组相比，PKA 抑制剂 H89 显著逆转了急性应激对小鼠棕色脂肪组织中 p-PKA substrate、p-CREB、KLF7、IL-6 表达的促进作用；显著逆转了急性应激后小鼠血清 IL-6 含量升高、血糖升高等表型；也显著逆转了急性应激对小鼠肝脏组织中 *Pck1* 等糖异生相关基因表达的促进作用。以上差异具有统计学意义(P<0.05)。在腹腔注射 ADRB3 激动剂 CL316,243 直接激活 ADRB3 的小鼠模型中，得到与上述一致的结果。

3.2、ADRB3 激活可通过 cAMP-PKA-CREB 通路上调棕色脂肪细胞 KLF7 和 IL-6 的表达

(1) 与单纯 CL316,243 处理组相比，PKA 抑制剂 H89 显著逆转了 ADRB3 激活对原代棕色脂肪细胞 p-PKA substrate、p-CREB、KLF7、IL-6 表达的上调作用。收集棕色脂肪细胞上清与小鼠肝细胞 Hepa 1-6 共培养 4h 后，与 CL316,243 处理组相比，H89 预处理的棕色脂肪细胞上清液显著抑制了 Hepa 1-6 细胞中糖异生相关基因 *Pck1*、*Gck*、*G6pc* 的 mRNA 表达水平。以上差异具有统计学意义(P<0.05)。在 C3H10T1/2 棕色脂肪细胞中得到与上述一致的结果。

(2) 腺苷酸环化酶激活剂Forskolin(可增加cAMP浓度)处理原代培养的小鼠棕色脂肪细胞4 h后，细胞中 p-PKA substrate、p-CREB、KLF7、IL-6 的表达水平显著增加。收集细胞上清液与小鼠肝细胞Hepa 1-6共培养4 h后，Forskolin处理的棕色细胞上清液显著促进了Hepa 1-6糖异生相关基因 *Pck1*、*Gck*、*G6pc* 的表达水平。PKA 抑制剂 H89 预处理原代培养的小鼠棕色脂肪细胞 4 h，可显著逆转 Forskolin 引起的上述变化。以上差异具有统计学意义(P<0.05)。在 C3H10T1/2 棕色脂肪细胞中得到与上述一致的结果。

4. CREB 可靶向调控 *KLF7*

利用 NCBI 数据库查找人 *KLF7* 基因启动子区序列，并运用转录因子结合位点信息数据库 JASPAR database 预测后发现：转录因子 CREB 与 *KLF7* 启动子区存在多个结合位点。双荧光素酶报告基因实验结果显示，过表达 CREB 可显著增加 *KLF7* 启动子区的荧光素酶活性值。收集 CREB 过表达的 HEK-293T 细胞样本进行染色质免疫共沉淀实验，qRT-PCR 结果显示，CREB 与 *KLF7* 启动子区结合位点为-88~-81、-966~-959、-1894~-1887、-1963~-1956。以上差异具有统计学意义(P<0.05)。

结论：

急性应激状态下，ADRB3 激活后可通过 cAMP-PKA-CREB-KLF7 途径上调棕色脂肪细胞 IL-6 的表达，促进肝脏糖异生。

关键词：急性应激；ADRB3；cAMP-PKA-CREB；KLF7；IL-6

论文类型：A (基础研究)

Abstract

Object:

Recent studies showed that ADRB3 activation during acute stress, enhances hepatic gluconeogenesis by promoting the production and release of IL-6 in brown adipocytes, powering the body to cope with stress. However, the specific molecular mechanisms that IL-6 production and release after ADRB3 activation have not been reported. ADRB3 activation plays an important role in adipose tissue thermogenesis, and adipolysis via the cAMP-PKA-CREB pathway during glucose uptake. Previous studies have found that the transcription factor KLF7 targets and upregulates the expression of IL-6 in adipocytes. Bioinformatics prediction suggested multiple binding sites for CREB in the *KLF7* promoter region. Based on the construction of a mouse model of acute stress and ADRB3 activation, as well as the in vitro culture of brown adipocytes and mouse hepatocytes, this study clarified whether the expression of IL-6 in brown adipocytes was regulated by the cAMP-PKA-CREB-KLF7 signaling pathway after ADRB3 activation under acute stress, which will provide a new theoretical basis for elucidating and enriching the new functions of brown adipocytes in acute stress.

Method:

1. Group and testing index of animal experiments

(1) Group of experimental animals: 6 week C57BL/6 male mice (n =40) was divided into control group (n=10), acute stress group (n=10), acute stress+ADRB3 antagonist group (n=10) and acute stress+PKA inhibitor group (n=10); 6 week C57BL/6 male mice (n =40) was divided into control group (n=10), ADRB3 agonist group (n=10), ADRB3 agonist+ADRB3 antagonist group (n=10) and ADRB3 agonist+PKA inhibitor group (n=10).

(2) Treatment of experimental animals: In the acute stress group, the control group no treated; The ADRB3 agonist group received an intraperitoneal injection of 1 mg/kg CL316,243 for 2 h, and the control group was injected with an equivalent amount of normal saline intraperitoneally; Mice in the ADRB3 antagonist group were pretreated by intraperitoneal injection with 10 mg/kg SR59203A for 1 h, and the control group received an equivalent dose of solvent (saline + 5% DMSO); In the PKA inhibitor group, mice were

pretreated with 5 mg/kg H89 for 1 h, and the control group was given an equal amount of solvent (saline + 5% DMSO).

(3) Sample collection and detection indicators: the general condition of mice in the above groups was monitored dynamically. Serum, brown adipose tissue and liver were collected. The expression level of IL-6 in serum was determined by Elisa. The expression of p-PKA substrate, p-CREB, KLF7 and IL-6 in mice brown adipose tissue were detected by qRT-PCR and Western Blot. Glucose tolerance test and the insulin tolerance test were performed to evaluate glucose tolerance and insulin sensitivity in mice. Pyruvate tolerance test was performed to evaluate the gluconeogenesis capacity in mice.

2. Brown adipocytes culture, treatment, and detection index

(1) Primary brown adipocytes and mouse fibroblast line C3H10T/2 was cultured and induced as mature brown adipocytes. Lipid droplets identified by oil red O staining, and brown fat biomarkers identified by qRT-PCR and Western Blot.

(2) Primary brown adipocytes and C3H10T1/2 were treated as follows: the ADRB3 agonist treated group for 5 μM CL316,243 for 4 h, and the equal amount of saline for the control group; the adenylate cyclase activator-treated group was treated with 10 μM Forskolin for 4 h, and the control group for equal amount of DMSO; the PKA inhibitor-treated group for 10 μM H89 for 4 h, and the control group for the equal amount of DMSO.

(3) Mouse hepatocytes Hepa 1-6 were treated as follows: medium from CL316,243 or Forskolin-treated brown adipocytes were collected with 2 μg/mL of IL-6 neutralizing antibody or isotype control, and samples were collected after 4 h of coculture with Hepa 1-6.

(4) Detection index: the mRNA expression levels of KLF7 and IL-6 were detected by qRT-PCR in primary cultures and C3H10T1/2 induced mature brown adipocytes. mRNA expression levels of *Pck1*, *Gck*, *G6pc*, and protein expression levels of p-PKA substrate, p-CREB, KLF 7 and IL-6 by Western Blot; and IL-6 in cell supernatants by Elisa kit.

3. Bioinformatics prediction and validation

The sequence of the of *KLF7* promoter region was searched by the NCBI database, and the JASPAR database was used to predict that there were multiple binding sites between the transcription factor CREB and the *KLF7* promoter region. After overexpression of CREB in 293T cells for 48 h, dual luciferase reporter assay showed that overexpression of CREB

significantly increased the luciferase activity value in the promoter region of KLF7. ChIP assay were performed to verify the targeted regulation of *KLF7* by CREB.

4. Statistical method

Data analysis was performed using SPSS 26.0. Firstly, the *t*-test was used for the two sets of data with normal distribution, the rank sum test was used for the two sets of data without normal distribution, and the multi-group data with normal distribution. Differences were considered statistically significant at $P<0.05$.

Results:

1. ADRB3 activation after acute stress upregulates the expression of KLF7 and IL-6 in brown adipocytes

1.1. The expression of KLF7 and IL-6 was increased in brown adipose tissue after ADRB3 activation, and promoted hepatic gluconeogenesis

The mouse model for ADRB3 activation: one was to construct a acute stress mouse model by retro-orbital bleeding to activate ADRB3; The other was to give mice intraperitoneal injection of ADRB3 agonist CL316,243 directly activate ADRB3, respectively, as follows.

Four hours after acute stress with retro-orbital bleeding, the protein expression level of p-PKA substrate and p-CREB in the brown adipose tissue of ADRB3 were significantly increased compared with the control group. At the same time, the mRNA and protein expression levels of KLF7 and IL-6 in brown adipose tissue of stressed mice were significantly elevated, the content of IL-6 in serum was significantly increased, and the blood glucose level was significantly increased, and acute stress had no significant effect on GTT and ITT in mice. The mRNA expression levels of *Pck1, G6pc,* and other gluconeogenic genes in the liver tissues of acutely stressed mice were significantly elevated, and the ability of gluconeogenesis in stressed mice was significantly enhanced, while acute stress had no significant effect on renal gluconeogenesis in mice. After intraperitoneal injection of ADRB3 antagonist SR59203A, the above phenotype was significantly reversed in stressed mice. Results consistent with those described above were obtained in a mouse model in which ADRB3 was directly activated by intraperitoneal injection of the ADRB3 agonist CL316,243. The above differences were statistically significant ($P<0.05$).

1.2. Differentiation of primary adipocytes and C3H10T1/2 mouse preadipocytes

The primary adipocytes were isolated from brown adipose tissue in the scapular region of 2-4 weeks of mice, and C3H10T1/2 preadipocytes were cultured in vitro. Under the

microscope, the preadipocytes were observed to be spindle-shaped, and after 2 days of adding inducer I for 2 days, the cell morphology became rounded and the cytoplasm became rough. After 6 days of addition of inducer II, a large number of small round lipid droplets aggregated in brown adipocytes. After 8 days of induction, a large number of lipid droplets were stained with red by oil red O, and the mRNA expression levels of thermogenesis-related genes Ucp1, Pgc1-α, and Ppar-α were significantly increased, indicating that brown adipocytes were successfully induced. The above differences were statistically significant (P<0.05).

1.3. ADRB3 activation significantly promoted the expression of KLF7 and IL-6 in brown adipocytes

The above two brown adipocyte models were used to perform the following experiments:

After treating primary mouse brown adipocytes with ADRB3 agonist CL316,243 for 4 h, compared with the control group, the downstream signaling molecule of ADRB3, the protein expression levels of p-PKA substrate and p-CREB in brown adipocytes were significantly elevated, while the mRNA and protein expression levels of KLF7 and IL-6 in brown adipocytes were significantly increased, and the content of IL-6 in cell supernatant was significantly increased. The brown adipocyte supernatant was collected and co-cultured with mouse hepatocytes Hepa 1-6 for 4 h, and it was found that the adipocyte supernatant treated with CL316,243 significantly promoted the mRNA expression of hepatocyte gluconeogenes-related genes *Pck1*, *Gck* and *G6pc*, while the use of 2 μg/mL IL-6 neutralizing antibody significantly reversed the above effect of CL316,243. Results consistent with the above were obtained in C3H10T1/2-induced brown adipocytes. The above differences were statistically significant (P<0.05).

2. Activation of ADRB3 promotes IL-6 expression in brown adipocytes via KLF7

After 48 h of transfection of si-KLF7 into primary brown adipocytes, the mRNA levels of IL-6 in primary brown adipocytes and the protein expression of IL-6 in cell culture supernatants were significantly decreased compared with the control group. After 48 h of transfection of KLF7 overexpression plasmid into primary brown adipocytes, KLF7 overexpression significantly promoted the mRNA expression level of IL-6 in primary brown adipocytes and the protein expression level of IL-6 in the cell culture supernatant compared with the NC group. Compared with CL316,243 group, transfection with si-KLF7 while

CL316,243 treatment significantly reversed the up-regulation of IL-6 expression in primary brown adipocytes. The above differences were statistically significant (P<0.05).

3. Expression of brown adipocytes KLF7 and IL-6 was up-regulated through the cAMP-PKA-CREB signaling pathway after ADRB3 activation

3.1. PKA inhibitors reversed the up-regulation of KLF7 and IL-6 expression in brown adipose tissue by acute stress

Compared with the control group, 5 mg/kg PKA inhibitor H89 significantly reversed the effect of acute stress on the p-PKA substrate, p-CREB protein expression, and the mRNA and protein expression of KLF7 and IL-6 in brown adipose tissue of mice. It also reversed the phenotypes of increased serum IL-6 content and increased blood glucose in mice after acute stress. Similarly, the PKA inhibitor H89 also significantly reversed the effect of acute stress on the mRNA expression of *Pck1,* and other gluconeogenic genes in mouse liver tissue. Results consistent with those described above were obtained in a mouse model in which ADRB3 was directly activated by intraperitoneal injection of the ADRB3 agonist CL316,243. The above differences were statistically significant (P<0.05).

3.2. After ADRB3 activation, KLF7 and IL-6 expression in brown adipocytes was regulated by the cAMP-PKA-CREB pathway

Two brown adipocyte models were used, one was primary cultured mouse brown adipocytes; the other is the mature brown adipocytes induced by the mouse preadipocyte line C3H10T1/2, and the following experiments were performed:

(1) After the PKA inhibitor H89 was pretreated with primary cultured mouse brown adipocytes for 4 hours, and the ADRB3 agonist CL316,243 was continued to be treated for 4 hours, H89 significantly reversed the up-regulation of ADRB3 activation on the expression of p-PKA substrate and p-CREB, as well as the up-regulation of KLF7 and IL-6 mRNA and protein expression compared with CL316,243 group. At the same time, after the brown adipocyte supernatant was collected and co-cultured with mouse hepatocytes Hepa 1-6 for 4 h, compared with the CL316,243 treatment group, the H89 pretreated brown adipocyte supernatant significantly inhibited the mRNA expression levels of gluconeogenes-related genes *Pck1*, *Gck* and *G6pc* in Hepa 1-6 cells. Results consistent with the above were obtained in mature brown adipocytes induced by the mouse preadipocyte line C3H10T1/2. The above differences were statistically significant (P<0.05).

(2) After 4 h treatment of primary brown adipocytes with adenyl cyclase activator Forskolin (which can increase the concentration of cAMP), the protein expression levels of

p-PKA substrate and p-CREB in the cells were significantly increased, and the mRNA and protein expression levels of KLF7 and IL-6 were significantly increased. After collecting cell supernatants and co-culturing with mouse hepatocytes Hepa 1-6 for 4 h, the brown adipocytes supernatants treated with Forskolin significantly promoted the mRNA expression levels of Hepa 1-6 gluconeogenes-related genes *Pck1*, *Gck*, and *G6pc*. After pretreatment of primary brown adipocytes with the PKA inhibitor H89 for 4 h, and continued treatment of cells with Forskolin for 4 h, H89 significantly reversed the above changes caused by Forskolin. Results consistent with the above were obtained in mature brown adipocytes induced differentiation by the mouse preadipocyte line C3H10T1/2. The above differences were statistically significant (P<0.05).

4. CREB targeted regulate *KLF7*

The sequence of the promoter region of human *KLF7* gene was searched in the NCBI database, and the JASPAR database software was used to predict that there were multiple binding sites between the transcription factor CREB and the promoter region of *KLF7*. The results of dual luciferase reporter assay in HEK-293T cells showed that overexpression of CREB could significantly increase the luciferase activity of KLF7. Samples of HEK-293T cells overexpressing CREB were collected for Chromatin Immunoprecipitation, and qPCR results showed that the binding sites -88~-81, -966~-959, -1894~-1887, and -1963~-1956. were significantly enriched. The above differences were statistically significant (P<0.05).

Conclusion:

Activation of ADRB3 after acute stress up-regulates the expression of IL-6 in brown adipocytes through the cAMP-PKA-CREB-KLF7 pathway, and promotes hepatic gluconeogenesis.

Key words: acute stress; ADRB3; cAMP-PKA-CREB; KLF7; IL-6

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