C-type Natriuretic Peptide Promotes Adipogenic Differentiation of Goat Adipose Derived Stem Cells via cGMP/PKG/ p38 MAPK Signal Pathway

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Research

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Abstract

Background

Two types of adipose tissue, white adipose and brown adipose, have been identified in mammals. For goat, adipose tissue also plays an important role in improving meat and milk quality. C-type natriuretic peptide (CNP) is a member of natriuretic peptide family. Once CNP binds to natriuretic peptide receptor B (NPR-B), NPR-B induces the production of cGMP, thereby activating PKG and downstream targets. The expression of CNP and NPR-B in adipose tissue led to a hypothesis that CNP could have roles involving in regulation of adipogenesis in goat. However, there are few studies on the relationship between CNP and adipogenesis in goat. In the present study, goat adipose derived stem cells (ADSCs) were isolated and employed to investigate the effect of CNP on adipogenesis in goat.

Results

During adipogenic differentiation in goat ADSCs, the expression of NPR-B increased at 4 d and 8 d and reduced at 12 d after differentiation. When goat ADSCs were treated with 100 nM and 1000 nM CNP during adipogenic differentiation, there are more accumulation of lipid droplets in the cytoplasm and the up-regulation in the expression of adipogenesis relative genes including PPAR- γ , FASN and LPL. Interestingly, the expression level of brown adipose genes UCP-1 and PGC-1 α were also up-regulated. Furthermore, the cGMP level was increased after treatment with CNP. Adding cGMP analogues 8-pCPT-cGMP (PKG activator) could increase adipogenesis efficiency, and adding both PKG inhibitor Rp-8-CPT-cGMP and CNP inhibited adipogenic differentiation. After treatment with CNP or PKG activator, the phosphorylation of p38 MAPK, MK2 and ATF2 were up-regulated, and their phosphorylation were significantly inhibited when CNP and PKG inhibitor were added simultaneously. SB203580 is the specific inhibitor for p38 MAPK. After treatment with CNP and SB203580 simultaneously, the adipogenic differentiation efficiency of goat ADSCs was significantly inhibited, and the expression of UCP-1 and PGC-1 α were also reduced.

Conclusions

CNP could promote adipogenic differentiation of goat ADSCs. The stimulative effect of CNP on adipose differentiation depends on the cGMP/PKG/ p38 MAPK signal pathway. Our study will be helpful to understand the regulation mechanism of goat adipose differentiation, especially brown adipose differentiation.

Background

Adipose tissue is not only energy storage organ but also an important endocrine tissue in mammalian animal[1]. There are two adipose tissue including white adipose and brown adipose have been recognized[2]. White adipose is responsible for energy storage, instead, brown adipose is the main source of non-shivering thermogenesis[3]. For ruminants, moderate adipose tissue content is also relative to

meat production and quality[4]. Therefore, the investigation about regulative mechanism of adipose tissue development is necessary for improving cold tolerance and meat quality of goat. Adipose derived stem cells (ADSCs) are adult stem cells derived from adipose tissue characterising their specific cell surface markers and trilineage differentiation potentials[5]. Up to now, ADSCs have been successfully isolated from many livestock, including goat[6-8], sheep[9] and bovine[10-11]. ADSCs are easy to isolate, culture and induce adipogenic differentiation, providing a useful cell model to study the regulation of adipogenesis and energy metabolism.

Among many transcription factors related to adipogenic differentiation, peroxisome proliferator-activated receptor gamma (PPAR- γ) is considered to be the primary initiator and a prerequisite for adipogenic differentiation[12]. On the other hand, the second messengers cAMP and cGMP have been shown to be involved in adipogenic differentiation[13]. cAMP signaling via cAMP-dependent protein kinase (PKA) is important to both adipogenesis and lipolysis in white adipose tissue[14]. For brown adipose tissue, activation of β -adrenergic receptors(β -ARs), increasing cAMP levels and activating PKA, results in promotion of lipolysis[15]. Because PKA and cGMP-dependent protein kinase (PKG) share related motifs for phosphorylation of substrate, cGMP signaling is also essential for normal adipogenic differentiation and thermogenesis[16]. Treatment of preadipocytes 3T3-L1 with cGMP resulted in increase in expression of PPAR- γ and uncoupling Protein 1 (UCP1) which is an important regulator of adaptive thermogenesis and exclusively expressed in brown adipose tissue [17].

C-type natriuretic peptide(CNP) is a member of natriuretic peptide family, which plays unique and distinctive roles within the cardiovascular system. CNP exerts its biological effects via the activation of surface receptor, natriuretic peptide receptor B (NPR-B, also termed guanylyl cyclase-B, GC-B). NPR-B is a guanylyl cyclase receptor. Once CNP binds to NPR-B, NPR-B induces the production of cGMP, thereby activating PKG and downstream targets[18]. During induced adipogenesis in 3T3-L1, 3-isobutyl-1-methyl xanthine (IBMX), which is a non-specific phosphodiesterase inhibitor, could be replaced by CNP to maintain a relative high level of cGMP[19]. Since CNP can induce the formation of cGMP, it is interesting to study the effect of CNP on adipogenesis. Although CNP and NPR-B were only reported mainly expressed in lung, heart, ovary and uterus in goat[20-22], in human and rodents, CNP and NPR-B are expressed in adipose tissue and regulate energy metabolism[23-25]. Such studies have led to a hypothesis that CNP could have roles involving in regulation of adipogenesis in goat. However, as an important domestic animal, there are few studies on the relationship between CNP and adipogenesis in goat. In the present study, goat ADSCs were isolated and employed to investigate the effect of CNP on adipogenesis in goat.

Methods

Media and chemicals

Cell culture medium and supplements were purchased from Thermo Fisher Scientific (Fisher Scientific, Pittsburgh, PA) unless otherwise noted. Animal experimental procedures were carried out according to

protocols approved by the animal ethical committee of Northwest A&F University.

ADSCs isolation and culture

Goat ADSCs were isolated and cultured using methods previously reported with minor modifications (6). Briefly, 5 g of subcutaneous adipose tissue was harvested from the inguinal area of Guanzhong dairy goat. Adipose tissue was minced in PBS, and digested with same volume of PBS containing 0.2% collagenase type I at 37°C in a water-bath shaker for 60 min followed by centrifugation at 100g for 5 min. The pellet was resuspended in PBS and centrifugated at 100g for 5 min three times. The pellet finally was resuspended in medium consisting of Dulbecco's modified medium-F12, with 10 % fetal bovine serum and 100 I.U./mL penicillin, 100µg/mL streptomycin. The cells were seeded in a 60-mm Petri dish at a density of 2×10^5 cells/cm² and cultured at 37°C under 5% CO₂. Medium was changed every 3 d until the cells reached 80 % confluency. At 80% confluence, adherent cells were passaged by digestion with 0.25 % trypsin/EDTA.

Tri-lineage differentiation assays

Osteogenesis, chondrogenesis, and adipogenesis were performed according to ADSCs differentiation kit instructions (Cyagen Biosciences). Tri-lineage differentiation was visualized using cell-specific staining. Alizarin red staining, alcian blue staining, and oil-red-o staining, for osteogenesis, chondrogenesis, and adipogenesis, respectively were used.

Immunophenotyping by flow cytometry

Cells were harvested and fixed in 4% paraformaldehyde. After rinse three times with PBS, cells were incubated with fluorescein labeled antibodies (BD Biosciences) specific for adipose stem cells: CD29, CD44, CD90, CD105, as well as CD34 and CD45, all for 30 min at room temperature. Then, cells were rinsed three times with PBS and were assayed using a FACSCanto II flow cytometer (BD Biosciences), and the data were analyzed using a FLOWJO software (Tree Star, Inc).

Adipogenic differentiation and lipid droplet quantification

Cells were plated in 6-well plates at a density of 1×10^4 cells/cm² and cultured to 80% confluency. Then, growth medium was replaced with adipogenic induction medium: DMEM/F12, 10% FBS, 1 µM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 10 ng/mL insulin, and a compound of interest such as different concentrations of CNP, 100 nM CNP plus Rp-8-CPT-cGMP(PKG inhibitor), 100 nM CNP plus SB203580(p38 MAPK inhibitor), 8-PCT-cGMP(PKG activator) , SB203580. Cells were cultured for 12 d with medium changes every 2 d. After 12 d differentiation, samples were fixed in 4% paraformaldehyde for 20 min, and Oil Red O staining was performed for 20 min. The staining fluid was removed, followed by wash three times with PBS. Isopropyl alcohol was added to dissolve lipid drops, and the absorbance was measured at 550 nm using a microplate reader (Thermo Scientific™).

RNA extraction and cDNA synthesis

After 14 d differentiation, total RNA was extracted using RNAiso Plus (Takara) according to the manufacturer's instructions. 2 µg of total RNA was subsequently used for first-strand cDNA synthesis using PrimeScript™ RT reagent Kit (Takara). The quantitative RT-PCR reactions were performed with the Fast SYBR Green Master Mix (Genstar, China), data collection and data analysis were performed on the QuantStudio 6 Flex machine (Lifetechnologies, USA) by using the GraphPad Prism 6 software. Specific PCR primers were purchased from Sangong Biotech (Sangong Biotech, China), and the primer sequences were shown in Table 1. The PCR parameters were as follows: 95°C for 2 min, followed by 40 cycles each of 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s. Relative gene expression was obtained by normalizing with GAPDH expression, calculating differences in mRNA expression as fold changes relative to expression in 0 d differentiation group or 0 nM CNP group, for NPR-B expression or adipogenic genes respectively.

Western blot analysis

Cells were lysed with RIPA buffer (Solarbio) supplemented with 1 mM phenylmethylsulfonyl fluoride (Solarbio) on ice for 30 min. Western blot was carried out with equal amounts of protein. The primary antibodies were as follows: anti-β-actin (1:1000, 4970S, CST), anti-UCP-1 (1:500, ab23841, Abcam), anti-PGC-1α (1:500, ab54481, Abcam), anti-p-p38 MAPK (1:1000, 4631S, CST), anti-p38 MAPK (1:1000, 8690T, CST), anti-p-MK2 (1:1000, 3316T, CST), anti-MK2 (1:1000, 12155S, CST), anti-p-ATF2 (1:1000, 40749S, CST), anti-ATF2 (1:1000, 35031T, CST). The secondary antibodies used were goat anti-rabbit IgG-HRP (1:2000, ab205718, Abcam). The protein bands were detected with a chemiluminescence kit (Tanon, China).

Immunofluorescence

Cells were fixed for 30 min in 4% paraformaldehyde (Solarbio, China), permeabilized for 15 min in 0.2% TritonX-100 (Sigma), incubated for 2 h in blocking buffer (3% BSA in PBS), and treated with primary antibody against NPR-B (1:100, ab139188, Abcam) overnight at 4°C. The secondary antibody used was Goat Anti-Rabbit IgG-Alexa Fluor® 488 (1:100, ab150077, Abcam). The nuclei were stained with DAPI (Beyotime, China) for 5 min at room temperature. Images were captured using a fluorescence microscope (Olympus, Japan).

Statistics

All quantitative data are presented as mean ± standard deviation (SD) with no less than three replicates for each experimental condition. Comparison between experimental and control groups were performed with Factorial ANOVA with the use of SPSS 10.0. A value of P < 0.05 was considered statistically significant.

Results

Characterisation of goat ADSCs

Similar to mesenchymal stem cells, the isolated goat ADSCs have fibroblast-like morphology, short spindle shape with low nuclear cytoplasmic ratio (Fig 1. A). Osteogenic differentiation of goat ADSCs was confirmed by alizarin red staining. After 14 days osteogenic differentiation, alizarin red staining result showed dark red mineralised bone nodules (Fig 1. B), indicating that goat ADSCs can be induced to differentiate into osteoblasts. After 21 d induction, chondrogenic differentiation was confirmed by Alcian blue staining. As showed in Fig 1. C, extracellular matrix deposition was observed (Fig 1. C), indicating that goat ADSCs could be induced to differentiate into chondrocytes. Adipogenic differentiation of goat ADSCs was confirmed by Oil Red-O staining. After 12 days adipogenic differentiation, a lot of round lipid droplets were present in the cytoplasm (Fig 1. D), indicating that goat ADSCs can be induced to differentiate into adipocytes. Flow cytometry results showed that goat ADSCs expressed surface markers such as CD29, CD44, CD105 and CD90, but not hematopoietic stem cells surface markers including CD34 and CD45. The above results indicated that the isolated goat ADSCs possessing typical mesenchymal stem cells morphology, ability of tri-lineage differentiation and expression of specific surface markers are in accordance with the criteria for the identification of ADSCs.

The expression of NPR-B in goat ADSCs and adipogenic differentiation

There are three natriuretic peptide receptors, including NPR-A, NPR-B and NPR-C. In goat ADSCs, NPR-B is the most expressed natriuretic peptide receptor. Its expression level is about 20 times more than that of NPR-A ($p < 0.05$) and about twice that of NPR-C ($p < 0.05$) (Fig 2. A). The expression of NPR-B in goat ADSCs was further confirmed by immunofluorescence (Fig 2. B). During adipogenic differentiation, the expression of NPR-B increased at 4 d and 8 d ($p < 0.05$). But at the terminal of adipogenic differentiation (12 d), the expression of NPR-B was down-regulated compared to that at 4 d and 8 d ($p < 0.05$) (Fig 2. C).

C-type natriuretic peptide improves adipogenic differentiation of goat ADSCs

Since the expression of NPR-B is up-regulated during adipogenic differentiation, as the ligand of NPR-B, it is speculated that CNP may be involved in the regulation of goat ADSCs adipogenic differentiation. In order to explore the role of CNP in goat ADSCs adipogenic differentiation, goat ADSCs were treated with different concentration CNP during adipogenic differentiation. As showed in Fig 3. A, adipogenic differentiation was significantly improved by treatment with 100 nM and 1000 nM CNP compared to 0 nM and 10 nM CNP groups. In 100 nM and 1000 nM CNP groups, a large number of lipid droplets accumulated in the cytoplasm, and the relative content of oil red O is evidently more than that of 0 nM and 10 nM CNP groups ($p < 0.05$) (Fig 3. B). Real-time PCR results showed that the expression level of adipogenesis related genes, such as peroxisome proliferators-activated receptor γ (PPAR- γ), fatty acid synthetase (FASN) and lipoprotein lipase (LPL) were increased after treatment with 100 nM and 1000 nM CNP (Fig 3. C). Interestingly, the expression level of thermogenesis related genes, so called brown adipose genes, such as uncoupling protein 1 (UCP-1) and peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α) were also up-regulated after treatment with 100 nM and 1000 nM CNP (Fig 3. D). In order to confirm these results, the protein expression of UCP-1 and PGC-1 α were tested by Western-blotting. The

increased protein expression of UCP-1 and PGC-1 α were observed in 100 nM and 1000 nM CNP groups (Fig 3. E).

Production of cGMP mediated by NPR-B is involved in adipogenic differentiation of goat ADSCs

NPR-B has guanylate cyclase activity. After treatment with 100 nM and 1000 nM CNP, the cGMP level was increased compared to 0 nM and 10 nM CNP groups ($p < 0.05$) (Fig 4. A). Furtherly, adding cGMP analogues 8-pCPT-cGMP (PKG activator) during adipogenic differentiation could increase adipogenesis efficiency, which is similar to the effect of 100 nM CNP addition (Fig 4. B). On the contrary, when protein kinase G (PKG) inhibitor Rp-8-CPT-cGMP (PKG inhibitor) and CNP were added simultaneously during adipogenic differentiation, PKG inhibitor abolished stimulative effect of CNP on adipogenic differentiation (Fig 4. B). Meanwhile, adding PKG activator or CNP during adipogenic differentiation could increase the relative content of oil red O, treatment with PKG inhibitor had the opposite effect (Fig 4. C). Predictably, the expression level of adipogenesis related genes (PPAR- γ , FASN, LPL) and thermogenesis related genes (UCP-1 and PGC-1 α) were up-regulated after treatment with 100 nM CNP or PKG activator (Fig 4. D, E). But, PKG inhibitor inhibited the expression of these genes induced by CNP (Fig 4. D, E). As similar as q-PCR results, the protein expression of UCP-1 and PGC-1 α were increased after treatment with 100 nM CNP or PKG activator, and were reduced after treatment with PKG inhibitor (Fig 4. F).

C-type natriuretic peptide activate p38 MAPK during adipogenic differentiation of goat ADSCs

p38 MAPK is the downstream target of PKG, therefore, the phosphorylation of p38 MAPK, and its downstream target MAPKAPK-2 (MK2) and ATF2 was detected. As showed in Fig 5, after treatment with 100 nM CNP or PKG activator, the phosphorylation of p38 MAPK, MK2 and ATF2 were up-regulated compared with control group (0 nM CNP). However, their phosphorylation were significantly inhibited when CNP and PKG inhibitor were added simultaneously (Fig 5).

C-type natriuretic peptide promoted adipogenic differentiation of goat ADSCs depended on p38 MAPK activity

Since CNP activated p38 MAPK during adipogenic differentiation of goat ADSCs, it is necessary to investigate whether CNP induced adipogenesis of goat ADSCs is depended on p38 MAPK activity. SB203580 is the specific inhibitor for p38 MAPK. After treatment with 100 nM CNP and SB203580 simultaneously, the adipogenic differentiation efficiency of goat ADSCs was significantly inhibited compared to 100 nM CNP group (Fig 6. A). Meanwhile, the oil red O stain results confirmed the inhibitory effect of SB203580 (Fig 6. B). Furthermore, if p38 MAPK activity was inhibited by SB203580, the expression level of adipogenesis related genes (PPAR- γ , FASN, LPL) were down-regulated compared to 100 nM CNP group (Fig 6. C). and both of the thermogenesis related genes (UCP-1 and PGC-1 α) and their proteins were also reduced (Fig 6. D, E).

Discussion

In addition to energy storage and endocrine functions, adipose tissue differentiation and accumulation in mammals are also important for reproductive success and the adaptation to lactation[26]. More interesting is the differentiation of brown adipose tissue, because of its unique role in obesity, diabetes and metabolic syndrome. Investigators have identified a variety of signaling pathways involved in regulating adipogenic differentiation, including bone morphogenetic proteins, Wnt, Hedgehog[27] and fibroblast growth factors[28-29]. Moreover, epigenetic regulation is also considered to be involved in adipogenic differentiation[30-31]. Comparing to other tissue derived mesenchymal stem cells, ADSCs are easy to induce adipogenic differentiation, providing a convenient cell model to study the regulation of adipogenesis. In present study, goat ADSCs were isolated firstly. Isolated goat ADSCs has similar morphology to other tissue derived mesenchymal stem cells. Their potential of tri-lineage differentiation and expression of specific surface markers are in accordance with the criteria for the identification of ADSCs[32].

There are three types of natriuretic peptide receptors: NPR-A, NPR-B and NPR-C. CNP has the highest affinity with NPR-B[33]. Because of the lack of report about the expression of natriuretic peptide receptors in goat adipose tissue, the expression of natriuretic peptide receptors is first detected in goat ADSCs. As shown in Figure 2, goat ADSCs mainly express NPR-B rather than NPR-A. During adipogenic differentiation, the expression of NPR-B increased at 4 d and 8 d and reduced at 12 d. This result is similar to mouse preadipocytes, in which NPR-B is the dominant natriuretic peptide receptor, and its expression is down-regulated in mature adipocytes[34]. Since the expression of NPR-B gradually increased in the early stage of adipose differentiation, and other reports also suggested that oleic and linoleic acid that could promote adipogenesis increased the expression of NPR-B[35], these results suggest that CNP/NPR-B signal pathway may be involved in the regulation of adipose differentiation in goat ADSCs. Indeed, in present study, goat ADSCs were treated with different concentration CNP during adipogenic differentiation. As showed in Fig 3, adipogenic differentiation was significantly improved by treatment with 100 nM and 1000 nM CNP. This conclusion was based on the more accumulation of lipid droplets in the cytoplasm and the up-regulation in the expression of PPAR- γ , FASN and LPL. Interestingly, the expression level of brown adipose genes UCP-1 and PGC-1 α were also up-regulated. UCP1 and PGC-1 α are exclusively expressed in brown adipose tissue and critical to non-shivering thermogenesis[36]. Kangawa K and colleagues reported that CNP overexpression in mature adipocytes could increase the expression of PGC-1 α [37]. Collins S and colleagues reported that BNP could induce the expression of UCP-1 in white adipocytes resulting in the so-called “browning” of white adipocytes[38]. However, there are few studies on the effect of CNP on directly induced differentiation of brown adipocytes from ADSCs. In this study, we can not distinguish whether CNP firstly induce white adipocytes differentiation from ADSCs, and then CNP induce “browning” of white adipocytes, or whether CNP can directly induce the differentiation of brown adipocytes from ADSCs.

Katafuchi T and colleagues first reported that CNP could replace 3-isobutyl-1-methyl xanthine (IBMX) to induce adipogenesis in 3T3-L1 preadipocytes[19]. IBMX is a non-specific phosphodiesterase inhibitor, which can increase the level of cGMP level in cells, resulting in promotion of adipogenesis. NPR-B has guanylate cyclase activity. CNP also elevated the production of cGMP via NPR-B. Therefore, the

hypothesis that CNP could promote adipogenesis in goat ADSCs via the production of cGMP needs to be further verified. In this study, the cGMP level was increased after treatment with CNP. Adding cGMP analogues 8-pCPT-cGMP (PKG activator) could increase adipogenesis efficiency, and adding both protein kinase G (PKG) inhibitor Rp-8-CPT-cGMP (PKG inhibitor) and CNP inhibited adipogenic differentiation. These results suggest that cGMP, the second messenger, plays an important role in inducing adipocyte differentiation. Do other natriuretic peptides that can induce cGMP synthesis have the same effect? NPR-A is the receptor for both ANP and BNP. Similar to NPR-B, NPR-A has guanylate cyclase activity. As expected, ANP and BNP were reported participate in promotion of adipogenesis [34,39]. These results proved that CNP promoted adipogenesis in goat ADSCs depended on the production of cGMP.

It is well elucidated that cAMP/PKA signal pathway plays important roles in adipogenesis and thermogenesis of brown adipocytes[14, 40]. Especially for brown adipocytes, activation of β -AR triggers a kinase cascade from PKA to p38 MAPK in mouse adipocytes to increase the expression of UCP-1(41-42). Because PKA and PKG share related motifs for phosphorylation of substrate, in present study, the phosphorylation of p38 MAPK, and its downstream target MK2 and ATF2 was detected. As showed in Fig 5, after treatment with 100 nM CNP or PKG activator, the phosphorylation of p38 MAPK, MK2 and ATF2 were up-regulated, and their phosphorylation were significantly inhibited when CNP and PKG inhibitor were added simultaneously. Similar to our results, ANP and BNP also up-regulated the phosphorylation of p38 MAPK, MK2 and ATF2 in human and mouse adipocytes[43]. Although p38 MAPK signal pathway was reported be involved in “browning” of white adipocytes[43-45], the roles of p38 MAPK in adipogenic differentiation from goat ADSCs needs further study. On other hand, since CNP activate p38 MAPK during adipogenic differentiation of goat ADSCs, it is necessary to investigate whether CNP induced adipogenesis of goat ADSCs is depended on p38 MAPK activity. SB203580 is the specific inhibitor for p38 MAPK. After treatment with 100 nM CNP and SB203580 simultaneously, the adipogenic differentiation efficiency of goat ADSCs was significantly inhibited, and the expression of UCP-1 and PGC-1 α were also reduced. These results proved that CNP induced adipogenesis of goat ADSCs is depended on p38 MAPK activity.

Conclusions

In the present study, goat ADSCs were isolated and employed to investigate the effect of CNP on adipogenesis in goat. We have proved that CNP/NPR-B signal pathway could promote adipogenic differentiation of goat ADSCs. The stimulative effect of CNP on adipose differentiation depends on the cGMP/PKG/p38 MAPK signal pathway. Our study will be helpful to understand the regulation mechanism of goat adipose differentiation, especially brown adipose differentiation, and will provide a new approach to goat cold-resistance breeding and improving meat quality.

Abbreviations

CNP C-type natriuretic peptide

NPR-B natriuretic peptide receptor B

ADSCs adipose derived stem cells

PPAR- γ peroxisome proliferators-activated receptor γ

FASN fatty acid synthetase

LPL: lipoprotein lipase

UCP-1 uncoupling protein 1

PGC-1 α peroxisome proliferator-activated receptor coactivator-1 α

PKG cGMP-dependent protein kinase

PKA cAMP-dependent protein kinase

p38 MAPK p38 mitogen-activated protein kinase

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data supporting our findings are included in the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Q.W. and M.F. conceived the study. M.F., Y.Y., Y.M., Y.Z., Z.Z. and X.Z performed the experiments. Q.W. and M.F. wrote the manuscript. Q.W. and B.M supervised the study and approved the final version. All authors

read and approved the final manuscript.

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Tables

Table 1 Primer sequences for the RT-PCR and q-PCR analyses

Gene	Primer sequences (5' → 3')	Product size, bp	Anneal T, °C	Source
GAPDH	F: TGCCCGTTCGACAGATAGC	145	60	XM_005680968.3
	R: ACGATGTCCACTTTGCCAGTA			
PPAR-γ	F: GCCCAGGTTTGCTGAATGTG	143	60	NM_001285658.1
	R: CTGCCTGAGGTCCGTCATTT			
LPL	F: GTGTCTGCAGGGTACACCAA	118	60	NM_001285607.1
	R: CCCCAAGGCTGTATCCCAAG			
FASN	F: CTTAACAGCACGTCCCCCAT	149	60	NM_001285629.1
	R: TCCTCGGGCTTGTCTTGTTT			
UCP-1	F: CGCGCTTGTTTAAGGGACTC	132	60	XM_018061376.1
	R: CGCCGTCTACTCTCTACCCT			
PGC-1α	F: ATTGGCGTCATTCAGGAGCTG	198	60	NM_001285631.1
	R: TGGTCACTGCACCACTTGAG			

Figures

Figure 1

Characterisation of goat ADSCs. A, Morphology of the isolated goat ADSCs (passage 5) ; B, Osteogenic differentiation of goat ADSCs was confirmed by alizarin red staining; C, Chondrogenic differentiation of goat ADSCs was confirmed by Alcian blue staining. D, Adipogenic differentiation of goat ADSCs was confirmed by Oil Red-O staining; E, Flow cytometry results showed that goat ADSCs express surface

markers such as CD29, CD44, CD105 and CD90, but not hematopoietic stem cells surface markers including CD34 and CD45. Bar=100μm.

Figure 2

The expression of NPR-B in goat ADSCs and adipogenic differentiation. A, q-PCR analysis of NPR-A, NPR-B and NPR-C in goat ADSCs. Data are presented as the mean $\pm$ SEM of three independent experiments. *: $P < 0.05$; B, Staining of goat ADSCs (passage 5) for NPR-B. Bar=100μm; C, q-PCR analysis of NPR-B during adipogenic differentiation of goat ADSCs. Data are presented as the mean $\pm$ SEM of three independent experiments. *: $P < 0.05$.

Figure 3

C-type natriuretic peptide improves adipogenic differentiation of goat ADSCs. A, Representative images of adipogenic differentiation in goat ADSCs treated with different concentration CNP(0 nM, 10nM, 100nM, 1000nM). Bar=200μm; B, The relative content of oil red O in adipocytes differentiated from goat ADSCs treated with different concentration CNP. Data are presented as the mean $\pm$ SEM of three independent experiments. *: $P < 0.05$; C, q-PCR analysis of PPAR- γ , FASN and LPL in adipogenic differentiation in goat

ADSCs treated with different concentration CNP. Data are presented as the mean $\pm$ SEM of three independent experiments. *: $P < 0.05$; D, q-PCR analysis of UCP-1 and PGC-1 α in adipogenic differentiation in goat ADSCs treated with different concentration CNP. Data are presented as the mean $\pm$ SEM of three independent experiments. *: $P < 0.05$; E, Western blot assay showing the protein expression of UCP-1 and PGC-1 α in adipogenic differentiation in goat ADSCs treated with different concentration CNP.

Figure 4

Production of cGMP mediated by NPR-B is involved in adipogenic differentiation of goat ADSCs. A, Elisa assay of cGMP in goat ADSCs treated with different concentration CNP(0 nM, 10nM, 100nM, 1000nM). Data are presented as the mean $\pm$ SEM of three independent experiments. *: $P < 0.05$; B, Representative

images of adipogenic differentiation in goat ADSCs treated with 0 nM CNP, 100 nM CNP, 200μM PKG activator, 100 nM CNP and 200μM PKG inhibitor. Bar=200μm; C, The relative content of oil red O in adipocytes differentiated from goat ADSCs treated with 0 nM CNP, 100 nM CNP, 200μM PKG activator, 100 nM CNP and 200μM PKG inhibitor. Data are presented as the mean ± SEM of three independent experiments. *: P < 0.05; D, q-PCR analysis of PPAR-γ, FASN and LPL in adipogenic differentiation in goat ADSCs treated with 0 nM CNP, 100 nM CNP, 200μM PKG activator, 100 nM CNP and 200μM PKG inhibitor. Data are presented as the mean ± SEM of three independent experiments. *: P < 0.05; E, q-PCR analysis of UCP-1 and PGC-1α in adipogenic differentiation in goat ADSCs treated with 0 nM CNP, 100 nM CNP, 200μM PKG activator, 100 nM CNP and 200μM PKG inhibitor. Data are presented as the mean ± SEM of three independent experiments. *: P < 0.05; F, Western blot assay showing the protein expression of UCP-1 and PGC-1α in adipogenic differentiation in goat ADSCs treated with 0 nM CNP, 100 nM CNP, 200μM PKG activator, 100 nM CNP and 200μM PKG inhibitor.

Figure 5

C-type natriuretic peptide activate p38 MAPK during adipogenic differentiation of goat ADSCs. Western blot assay showed the protein expression and phosphorylation of p38 MAPK, MK2 and ATF2 in adipogenic differentiation in goat ADSCs treated with 0 nM CNP, 100 nM CNP, 200μM PKG activator, 100 nM CNP and 200μM PKG inhibitor.

Figure 6

C-type natriuretic peptide promoted adipogenic differentiation of goat ADSCs depended on p38 MAPK activity. A, Representative images of adipogenic differentiation in goat ADSCs treated with 0 nM CNP, 100 nM CNP, 10μM SB203580, 100 nM CNP and 10μM SB203580. Bar=200μm; B, The relative content of oil red O in adipocytes differentiated from goat ADSCs treated with 0 nM CNP, 100 nM CNP, 10μM SB203580,

100 nM CNP and 10 μ M SB203580. Data are presented as the mean $\pm$ SEM of three independent experiments. *: P < 0.05; C, q-PCR analysis of PPAR- γ , FASN and LPL in adipogenic differentiation in goat ADSCs treated with 0 nM CNP, 100 nM CNP, 10 μ M SB203580, 100 nM CNP and 10 μ M SB203580. Data are presented as the mean $\pm$ SEM of three independent experiments. *: P < 0.05; D, q-PCR analysis of UCP-1 and PGC-1 α in adipogenic differentiation in goat ADSCs treated with 0 nM CNP, 100 nM CNP, 10 μ M SB203580, 100 nM CNP and 10 μ M SB203580 . Data are presented as the mean $\pm$ SEM of three independent experiments. *: P < 0.05; E, Western blot assay showing the protein expression of UCP-1 and PGC-1 α in adipogenic differentiation in goat ADSCs treated with 0 nM CNP, 100 nM CNP, 10 μ M SB203580, 100 nM CNP and 10 μ M SB203580.