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Functional characterization of the promoter of carbonyl reductase 1 gene in porcine endometrial cells*

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Abstract: Prostaglandins (PGs) play a critical role in porcine reproduction, of which prostaglandin E2 (PGE2) and prostaglandin F2α (PGF2α) exert antiluteolytic and luteolysis actions, respectively. As a rate-limiting enzyme, carbonyl reductase 1 (CBR1) catalyzes the conversion of PGE2 to PGF2α. A high ratio of PGE2:PGF2α is beneficial to the establishment and maintenance of porcine pregnancy. PG is essential for the establishment of pregnancy which resembles the proinflammatory response and nuclear factor κB (NF-κB) is involved in the process. Bioinformatic analysis has shown that NF-κB is a possible factor bound to two *cis*-regulatory elements in *CBR1* promoter. In this study, we cloned the 2997 bp (−2875/+122) of the promoter, and constructed six 5'-deleted dual-luciferase reporter recombinant vectors. In endometrial cells, the region of P2 (−1640/+7) exhibited the greatest transcriptional activity at driving luciferase expression, but not significantly different from that of P1 (−2089/+7). The activity of P1, P2, and P3 (−1019/+7) was highly significantly higher than that of others (P<0.01), suggesting that two positive regulatory elements were likely present in the regions of −1640/−1019 and −1019/−647. The results also showed that the −1640/−647 region was indispensable for the promoter. The results of chromatin immunoprecipitation (ChIP) demonstrated that the NF-κB subunit p65 binds to one site around −1545/−1531. Using four reference genes, we found that the over-expression of *p65* enhanced the expression of *CBR1* (P<0.05) in porcine endometrial epithelial cells, while knockdown of the *p65* did not down-regulate the *CBR1* expression. These results indicated that NF-κB (p65) could bind to the special element of *CBR1* gene promoter in porcine endometrial epithelial cells in vitro. The binding site of NF-κB was a positive regulator for the *CBR1* gene promoter, but was not necessary for the basic expression.

Key words: Pig; Carbonyl reductase 1 (CBR1); Promoter; NF-κB; Endometrium
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1 Introduction

Prostaglandins (PGs) play a critical role in porcine reproduction. Prostaglandin F2α (PGF2α) is a luteolysis factor (Christenson *et al*., 1994), while prostaglandin E2 (PGE2) helps to establish and maintain early pregnancy by preventing luteolysis (Gadsby *et al*., 1993; Ziecik, 2002). Because of their opposite functions, the ratio of PGE2 to PGF2α is regulated accurately, which is helpful to maintain the porcine normal estrous cycle and pregnancy (Weems *et al*., 2006; Waclawik and Ziecik, 2007). Carbonyl reductase 1 (CBR1), known as prostaglandins 9-ketoreductase in pigs, catalyzes the conversion of

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PGE2 to PGF2α and modulates the PGE2:PGF2α ratio (Schieber *et al*., 1992; Tanaka *et al*., 1992; Ghosh *et al*., 2001; Waclawik and Ziecik, 2007). A low level of endometrial CBR1 facilitates a high PGE2:PGF2α ratio which is essential for the establishment and maintenance of porcine pregnancy (Waclawik and Ziecik, 2007). The higher ratio of PGE2:PGF2α may be a beneficial factor for sows that have large litter sizes (Bazer *et al*., 1991; Zhang *et al*., 2013). During the early pregnancy before implantation (11–12 d) of pig, elevated amounts of estrogen from conceptus decreased CBR1 protein expression and then increased PGE2 secretion and the PGE2: PGF2α ratio (Waclawik *et al*., 2009). Recent research showed that *CBR1* exhibited a lower expression level in peripheral blood from pregnant sows (14 d after insemination) than from non-pregnant sows, indicating the importance of the gene in early pregnancy (Shen *et al*., 2014).

In the mid-secretory phase of the estrous cycle, proinflammatory responses occur in the endometrium for preparation of implantation of the fertilized conceptus. Implantation resembles an inflammatory event and can be mediated via activation of the nuclear factor kappa B (NF-κB)-dependent gene transcription (Ross *et al*., 2010). In the porcine uterine endometrial epithelium, numerous genes including interleukin-6 (IL-6) and leukemia inhibitory factor are regulated by NF-κB (Geisert and Yelich, 1997). In humans, the promoter of prostaglandin E2 synthase (*PGES*) gene, which encodes the catalyzing enzyme for the conversion of PGH2 to PGE2, was reported to contain a functional estrogen response element (ERE) upstream of the transcription start site (Frasor *et al*., 2008), and estrogen and IL-1 (a proinflammatory cytokine) interact positively to enhance ERE activity in the endometrial epithelium and to up-regulate the expression of *PGES* (King *et al*., 2010). All these indicated that higher expression of *PGES* was mediated by NF-κB-dependent genes and lower CBR1 was helpful to keep a higher PGE2 synergistically for gestation establishment during the implantation window.

NF-κB is a heterogeneous collection of dimers consisting of five family members: NF-κB1 (p105/p50), NF-κB2 (p100/52), Rel A (p65), Rel B, and c-Rel (Lindström and Bennett, 2005). The heterodimer of p50 (NF-κB1):p65 (RelA) is the most abundant form (Ghosh *et al*., 1998). NF-κB was reported to be activated during peri-implantation in pigs (Mathew *et al*., 2011). Previous studies showed that the expression of *p65* and *p50* increased during the mid-secretory phase (Laird *et al*., 2000), and the mRNAs for *p65* and *p105* NF-κB subunits were reported to be maximally expressed in the human endometrium from the putative implantation window (King *et al*., 2010). In porcine ovarian cells, p50/p50 promoted the release of PGF2α but did not influence the release of PGE2, while p65/p65 enhanced the release of PGE2 and PGF2α (Pavlová *et al*., 2011).

The above results showed that the members of NF-κB played roles in different porcine tissues. We found that there was a putative binding site of NF-κB in the promoter of *CBR1* gene and speculated that the expression of the gene might be mediated by NF-κB. Considering the importance of *CBR1* in gestation establishment in early pregnancy, we aimed to detect the core region of the *CBR1* gene promoter and discover whether NF-κB might be involved in mediating the expression of *CBR1* in porcine endometrial cells.

2 Materials and methods

2.1 Sample collection and isolation of genomic DNA

All animal procedures in the present research were approved by the Animal Care and Use Committee of Guangdong Province, China. The ear tissues from Erhualian pigs were collected and preserved in 70% ethanol for DNA extraction. Genomic DNA was extracted from the ear tissues using the phenol method.

2.2 Cloning of porcine *CBR1* gene promoter and construction of 5'-deleted recombinant vectors

Primers are listed in Table 1. Based on the porcine *CBR1* gene sequence (Gene ID 397143), *CBR1*-5' primers were used to amplify the *CBR1* promoter of 2997 bp (−2875/+122). The fragment was cloned into the pMD18-T vector (Promega, USA) and sequenced. The cloned fragment was used as a template to amplify the series of *CBR1* promoter fragments. With the primers of p1, p2, p3, p4, p5, and p6, the series of fragments of the *CBR1* promoter, P1 (−2089/+7), P2 (−1640/+7), P3 (−1019/+7), P4 (−647/+7),

Table 1 Primers and siRNA sequences for the functional characterization of porcine *CBR1* gene promoter

Name	Sequence*	Location	Size (bp)
CBR1-5'	F: 5' GGAAAGTGGAATGGATGTCA 3'	−2875/+122	2997
	R: 5' AGTGTTGGAAGACATGGCTG 3'		
p1	F: 5' *CG*acgcgtCGTCGTCATGTGGAAAGGA 3'	−2098/+7	2106
	R: 5' *CCG*ctcgagCGGAGCCAGGTAGACCAGA 3'		
p2	F: 5' *CG*acgcgtCGAGACTGAGGAAAACTA 3'	−1640/+7	1648
	R: 5' *CCG*ctcgagCGGAGCCAGGTAGACCAGA 3'		
p3	F: 5' *CG*acgcgtCGAGATGTCCTGGCTACAC 3'	−1019/+7	1027
	R: 5' *CCG*ctcgagCGGAGCCAGGTAGACCAGA 3'		
p4	F: 5' *CG*acgcgtCGATAGGCATTTTCAGCAA 3'	−647/+7	655
	R: 5' *CCG*ctcgagCGGAGCCAGGTAGACCAGA 3'		
p5	F: 5' *CG*acgcgtCGAAGACAATGAATCTGC 3'	−334/+7	342
	R: 5' *CCG*ctcgagCGGAGCCAGGTAGACCAGA 3'		
p6	F: 5' *TT*acgcgtAGGGAGGGCAGAAGGC 3'	−80/+7	88
	R: 5' *CCG*ctcgagCGGAGCCAGGTAGACCAGA 3'		
ChIP-1539	F: 5' CAGCGCAAACATGGAAAT 3'	−1570/−1389	180
	R: 5' CCTAACCAAGGAAGGGAG 3'		
ChIP-869	F: 5' GAAGCTACTGTTTACCCTCAA 3'	−972/−753	220
	R: 5' ACAATCCCTGGCACCCT 3'		
p-p65	F: 5' *CGG*gatatcATGGACGACCTCTTCCC 3'		2880
	R: 5' *CGG*ggtaccTTAGGAGCTGATCTGACTCA 3'		
p-RPS20	F: 5' CGCTCCTGGCTCACCGCTGTT 3'		148
	R: 5' TGCGGCTGGTGAGGGTGATCC 3'		
18s	F: 5' AGGCCCTGTAATTGGAATGA 3'		150
	R: 5' CTCCCAAGATCCAACTACGA 3'		
GADPH	F: 5' CAGGTTGTGTCCTGTGACTT 3'		90
	R: 5' CTTGACGAAGTGGTCGTTGA 3'		
β-actin	F: 5' TGTTCGAGACCTTCAACACC 3'		285
	R: 5' AGCACAGCTTCTCCTTGATG 3'		
siRNA1	Sense: 5' GCACCGGAUUGAGGAGAAA dTdT 3'		
	Antisense: 3' dTdT CGUGGCCUAACUCCUCUUU 5'		
siRNA2	Sense: 5' CCAACACUGCAGAGCUCAA dTdT 3'		
	Antisense: 3' dTdT GGUUGUGACGUCUCGAGUU 5'		
siRNA3	Sense: 5' GAAGAAGAGUCCUUUCAAU 3'		
	Antisense: 3' dTdTCUUCUUCUCAGGAAAGUUA 5'		

* Italic letters show the protection bases; lowercase letters show *Mlu*I (acgcgt) and *Xho*I (ctcgag) sites for p1, p2, p3, p4, p5, and p6, and *Kpn*I (gatatc) and *Eco*RV (ggtacc) for p-p65

P5 (−334/+7), and P6 (−80/+7) were respectively amplified. The fragments were cloned into the pMD18-T vector and sequenced, and the positive vectors were digested with *Mlu*I and *Xho*I restrictive enzymes. The pGL-Basic vector (Promega, USA) was also digested with the same restrictive enzymes. Each digested promoter and vector fragment were linked with the T4 DNA ligation enzyme. The recombinants were detected by the above restrictive enzymes and sequenced. The endotoxin free plasmids were extracted with OMEGA GenExtract (USA).

CBR1 promoter alignments were performed using ClustalW2 (http://www.ebi.ac.uk). Putative transcription factor binding sites in the promoter region of the porcine *CBR1* gene were predicted using MatInspector (http://www.genomatix.de). The CpG islands in the promoter were analyzed using the tool available from http://www.urogene.org/methprimer/index1.html.

2.3 Culture of porcine endometrial epithelial cells

The culture of endometrial epithelial cells was based on the procedures in Zhang *et al.* (1991), Blitek and Ziecik (2004), and Blitek *et al.* (2007). The uterus of sows collected from a local abattoir was ligatured in the incision, placed in an ice box, and transported to the laboratory within an hour. The tissue was washed with 75% (v/v) ethanol and phosphate buffer solution (PBS) containing 100 IU/ml penicillin and 100 μg/ml streptomycin. The uterine horn was cut longitudinally, separated from the myometrium, and washed with PBS. The tissue was minced into 1 mm^3 and digested by 0.1% (1 g/L) collagenase I at 37 °C under shaking at 100 r/min for 2 h. Cell suspension was filtered to remove undigested tissue fragments, and pelleted at 500 r/min for 10 min. Red blood cells were lysed by a lysing buffer. The obtained epithelial cells were washed twice with PBS, and re-suspended in 1 ml of a culture medium (DMEM/F12 containing 10% (v/v) fetal bovine serum with 100 IU/ml penicillin and 100 μg/ml streptomycin). Cells were counted in a haemocytometer, plated in 25-cm^2 culture flasks at a density of 2×10^5 cells/ml, and incubated at 37 °C in a humidified incubator with 5% CO_2. After reaching confluence, cells were seeded onto 24-well plates for transfection and luciferase assays.

2.4 Transient transfections and luciferase activity assays

When epithelial cells were approaching confluence with 50%–80%, they were transfected by the constructed 5'-deleted recombinant vectors. The transfections were carried out using the Lipofectamine reagent (Invitrogen, USA) according to the manufacturer's instructions. Luciferase activity was determined with the dual-luciferase reporter assay system (Promega, USA).

2.5 Chromatin immunoprecipitation for the binding of NF-κB to the promoter

Chromatin immunoprecipitation (ChIP) procedures were performed with endometrial epithelial cells with the EZ-ChIP kit (Millipore, USA). The transcription factor p65 was immunoprecipitated by anti-p65 antibody (Abcam, UK). The anti-RNA polymerase II antibody and normal mouse IgG antibody were used as controls. Recovery and purification of DNA fragments from degradation of cross-linking DNA were used as the template for polymerase chain reaction (PCR). PCR was performed with primers ChIP-1539 and ChIP-869 for 180 and 220 bp, respectively, which contain the putative binding sites for NF-κB (−1545/−1531 and −875/−861 regions) (Table 1).

2.6 Over-expression of p65 on the acceleration *CBR1* gene promoter

With the primer of p-p65 (Gene ID: 100135665, Table 1), the 2880 bp of *p65* coding sequence (CDS) was cloned and was linked to pcDNA3.1/mcy-His(−)B. The over-expression vector of *p65* was constructed with restrictive enzymes of *Kpn*I and *Eco*RV. The vector was transfected into endometrial epithelial cells. After 48 h, the cells were washed twice with PBS. The cell mRNA was extracted with TRIzol (TaKaRa, Japan). The expression of *CBR1* and *p65* was detected by quantitative real-time PCR (qRT-PCR) using the genes of *18s* (AY265350.1), *β-actin* (DQ452569.1), *GAPDH* (KJ786424.1), and ribosomal protein S20 (*RPS20*) (Wang *et al.*, 2011) (NM_001129954, p-RPS20) as the reference genes (Table 1).

2.7 Knockdown of *p65* on the expression of *CBR1*

Three siRNAs (Ambion, UK) in Table 1 were designed to knockdown *p65* to further reveal the effect of NF-κB on the expression of the *CBR1* gene. The siRNAs were transfected into endometrial epithelial cells with Lipofectamine LTX and PLUS reagents (Invitrogen, USA) following the manufacturer's instructions. After the transfection, the expression of *CBR1* and *p65* was measured by qRT-PCR using the reference genes as above.

2.8 Statistical analysis

Data were analyzed by one-factor analysis of variance (ANOVA) and means were separated using the Tukey's test.

3 Results

3.1 Functional analysis of porcine *CBR1* promoter

The sequence of the 2997-bp fragment was amplified, and was submitted to GenBank (JQ743647), spanning −2875/+122 of the porcine *CBR1* promoter

and containing a region of 1067 bp (−2370/−1304) which was not part of the reference sequence (Gene ID: 397143). A search of putative *cis*-acting elements of porcine *CBR1* gene using MatInspector showed some typical regulatory elements, including GC-Box (−359/−353) and E-Box (−882/−860) identified, and putative binding sites for NF-κB (−1545/−1531, −875/−861), cAMP-response element-binding protein (CREB) (−617/−597), CCAAT/enhancer-binding protein (C/EBP) (−584/−560), and octamer transcription factor 1 (OCT-1) (−396/−382) predicted. No typical CpG islands were found in the sequence.

Based on these results, the luciferase activity driven by the six constructed 5'-deleted plasmids of the promoter was measured in endometrial cells (Fig. 1). P2 (−1640/+7) was the greatest at driving luciferase expression in all the constructs. The activity of P1 (−2098/+7) was the second highest, but not significantly different from P2 (P>0.05). P2 and P3 (−1019/+7) differed significantly (P<0.05), suggesting that a positive *cis*-regulatory element was likely present in the −1640/−1019 region. The activity of P1–P3 was highly significantly higher than that of others (P<0.01), which suggested that another positive regulatory element was likely present in the region of −1019/−647. Together, it was shown that the −1640/−647 region was indispensable for the *CBR1* promoter.

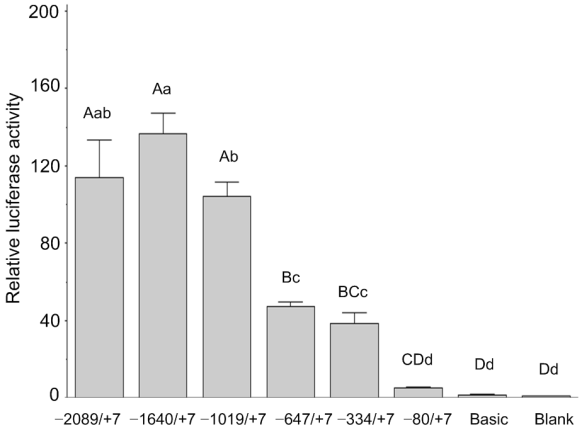
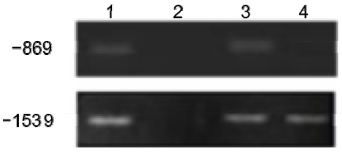


Fig. 1 Luciferase activity assays for the six fragments of the promoter in endometrial epithelial cells

Basic is the negative control with transfection of pGL3-basic vector, and Blank is the blank control without the transfection. Data were expressed as mean±standard error of mean (SEM) (n=3). Different uppercase and lowercase letters above the bar represent the difference at P<0.01 and P<0.05, respectively

3.2 Interaction of NF-κB on porcine *CBR1* core promoter

With MatInspector, two putative NF-κB binding sites were predicted in −1545/−1531 and −875/−861 regions in the *CBR1* core promoter. The ChIP results showed that p65 was bound to a site in the −1545/−1531 region but not in −875/−861 region (Fig. 2). Thus, it is highly likely that *CBR1* gene expression is directly mediated by NF-κB in porcine endometrial epithelial cells in vivo.



1. Input, DNA derived from samples prior to immunopreciptation;
2. IgG, negative control, the PCR result for the DNA immunoprecipitated with an IgG antibody;
3. RNA polymerase II, positive control, the PCR result for the DNA immunoprecipitated with a RNA polymerase II antibody;
4. Anti-p65, the PCR result for the DNA immunoprecipitated with a p65 antibody

Fig. 2 ChIP analysis of the *CBR1* promoter

3.3 Effect of NF-κB on porcine *CBR1* promoter

Gain and loss of *p65* in endometrial cells were examined to further detect the effect of NF-κB on *CBR1* expression. After the transfection into the cells for 48 h, the *p65* was over-expressed strongly, which significantly enhanced the expression of *CBR1* (P<0.05) compared with the negative control and the blank with *RPS20* as the reference gene (Fig. 3).

Then, three designed siRNAs were transfected into the cells' expression and the endogenous *p65* was nearly completely silenced by siRNA3 (Fig. 4). Under the silence of siRNA3, the mRNA of *CBR1* did not decrease in the cells compared with the four reference genes (*RPS20*, *18s*, *β-actin*, and *GADPH*) (Fig. 5).

4 Discussion

Many hormones are involved in the establishment and maintenance of porcine pregnancy. PG is an important hormone for its variety and multifunction. As two important members of PG, PGE2 is an anti-luteolytic factor (Gadsby *et al.*, 1993; Ziecik, 2002),

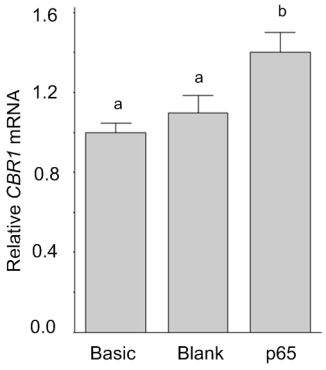


Fig. 3 Effect of p65 overexpression on *CBR1* mRNA level

Basic is the negative control with transfection of empty vector; Blank is the blank control without the transfection. Data are expressed as mean±SEM (n=3). Different letters above the bar show significant difference at P<0.05

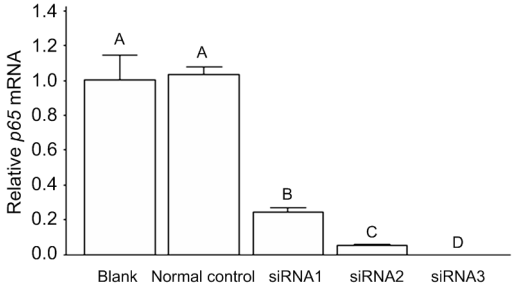


Fig. 4 *p65* expression under the silence of three siRNAs

Data are expressed as mean±SEM (n=3). Different letters above the bar show significant difference at P<0.01

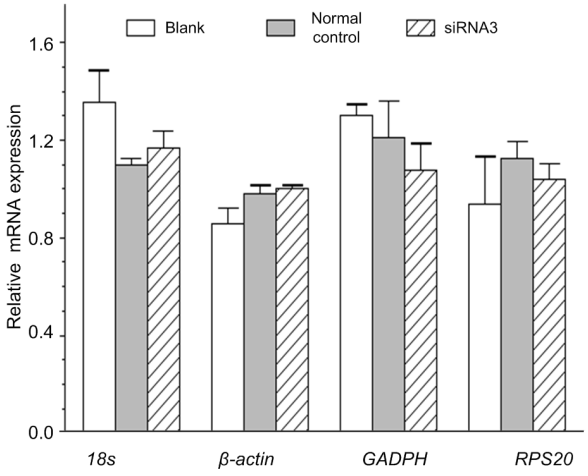


Fig. 5 *CBR1* expression with the four genes as references under the silence of siRNA3

Data are expressed as mean±SEM (n=3)

while PGF2α exerts luteolytic action (Christenson *et al.*, 1994). Thus, the ratio of PGE2 to PGF2α is regulated accurately. As a rate-limiting enzyme, *CBR1* catalyzes the conversation of PGE2 to PGF2α, and regulates the ratio according to physiologic conditions (Waclawik and Ziecik, 2007). The catalytic extent of *CBR1* will influence the ratio of the two members. So the regulation of *CBR1* gene expression is involved (Miura *et al.*, 2013; Guo *et al.*, 2014).

The establishment and maintenance of porcine pregnancy are dependent on the corpora luteum function. These events resemble proinflammatory responses. As an important inflammatory factor, NF-κB is a transcription factor that controls the expression of many reproduction-associated genes. The binding sites of NF-κB have also been found in the human *CBR1* promoter (Lakhman *et al.*, 2007). In human endometrial stromal and amnion mesenchymal cells, NF-κB was able to promote PGF2α production through oxygen radicals (Sugino *et al.*, 2004) and prostaglandin E syntheses (PTGES) (Ackerman *et al.*, 2008). On the contrary, NF-κB negatively regulated progesterone (P4) catabolism in rat corpus luteum (Telleria *et al.*, 2004). NF-κB exhibited opposing effects on different genes. In the PG synthesis pathway, the conversion of PGE2 to PGF2α is changed with estrous phases or tissues and regulated by *CBR1* (Waclawik *et al.*, 2009). However, whether NF-κB is a vital regulator for porcine *CBR1* is not well determined.

It is known that NF-κB binds specific sites (κB sites, 5'-GGGRNNYYCC-3', where R is purine, Y is pyrimidine, and N is any kind of nucleotide) of various genes and enhances gene expression (Inoue *et al.*, 2007). In the region of the porcine *CBR1* promoter, two putative similar binding sites were found in −1545/−1531 and −875/−861 regions, which locate in the core promoter region (−1640/+7; Fig. 1). We found that p65 bound with one assumed site of −1545/−1531 but another site of −875/−861 through ChIP in endometrial cells (Fig. 2). This showed that NF-κB may participate in the transcription of *CBR1*. For target gene transcription activation, NF-κB usually exerts its roles with p50/p65 heterodimer (Inoue *et al.*, 2007). In our work, over-expression of p65 enhanced the *CBR1* mRNA, which showed that p65 played a promotion role on *CBR1* (Fig. 3).

When *p65* was silenced by the siRNA3, we first detected that the *CBR1* gene expression was not influenced using the reference gene of *RPS20* which had been shown to be the best reference gene in the study of endometrial gene expression (Wang *et al.*, 2011). Then the other three common reference genes were used to verify the *CBR1* gene expression when the *p65* was silenced and the same results were obtained (Fig. 5). We concluded that the knockdown of *p65* will not decrease the mRNA of *CBR1*. From our results, it seemed that NF-κB would enhance the expression of *CBR1* and then decrease the ratio of PGE2 to PGF2α which would not be helpful to the establishment and maintenance of porcine pregnancy. However, the data showed that the levels of endometrial *CBR1* on gestation day 12 (GD12) and GD14 did not differ (Waclawik and Ziecik, 2007), and that the ratio of PGE2 to PGF2α on GD14 was higher than that on GD12 (Bazer *et al.*, 1991). It was speculated that other factors, such as CBR2, would play a role in the conversion of PGE2 to PGF2α (http://www.kegg.jp/pathway/hsa00590).

Methylation is mainly found in the CpG islands and decreases the transcription extent. Although the core *CBR1* promoter of humans had a typical CpG island (AP001724), we did not find CpG islands in the region of the porcine *CBR1* promoter.

The NF-κB was expressed during the porcine per-implantation period (Ross *et al.*, 2010), but the *CBR1* level was not increased (Waclawik and Ziecik, 2007). This may be related to post-transcription regulation. It is noted that hsa-miR-574-5p can effectively down-regulate human *CBR1* expression in the human lymphoblastoid cell (Kalabus *et al.*, 2012). Ssc-miR-574 expressed in porcine endometrial cells may reduce the *CBR1* level in porcine endometriun, and hence the expression p50:p65 did not differ significantly between GD10–GD17 ($P>0.05$) (Ross *et al.*, 2010), whereas *CBR1* mRNA and protein during GD10–GD13 were significantly lower than those of other days ($P<0.05$) (Waclawik and Ziecik, 2007). This showed that the stable expression p50:p65 did not keep the *CBR1* mRNA higher during GD10–GD17. During GD10–GD13, porcine *CBR1* gene may be decreased by negative factors or miRNAs.

In conclusion, we have shown that the porcine *CBR1* promoter region contained multiple *cis*-regulatoty elements. NF-κB enhances the *CBR1* expression but does not influence its basal expression in porcine endometrial epithelial cells. It seemed that NF-κB was not essential to the basal expression of *CBR1*. However, as a transcription factor, NF-κB does have an enhancing effect on *CBR1* expression.

Compliance with ethics guidelines

Ai-ling ZHANG, Xian-yue SUN, Qi YIN, Jian-hua ZENG, Zhe ZHANG, Jia-qi LI, and Hao ZHANG declared that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on animal research. All institutional and national guidelines for the care and use of laboratory animals were followed.

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中文概要

题　目：*CBR1* 基因启动子在猪子宫内膜细胞的功能研究

目　的：研究 *CBR1* 基因启动子在猪子宫内膜细胞的表达调控机制。

创新点：发现 *CBR1* 基因启动子在猪子宫内膜受到炎性因子核转录因子 kappa B（NF-κB）成员 p65 调控。p65 对该启动子具有正向调节作用，但是对于 *CBR1* 基因的表达并不是必需的。

方　法：通过双荧光素酶报告基因载体确定 *CBR1* 基因启动子转录活性区，通过染色质免疫沉淀（ChIP）技术确定 p65 能够结合 *CBR1* 基因启动子，通过超表达和干扰表达实验证实 p65 对 *CBR1* 基因启动子的调控作用。

结　论：猪 *CBR1* 基因启动子−1640/−647 区对于其转录活性是必需的，在−1545/−1531 区存在 p65 的结合位点。p65 在猪子宫内膜细胞中促进了 *CBR1* 基因 mRNA 的表达，但是干扰 p65 则不会造成 *CBR1* 基因 mRNA 表达量下降，推断 p65 不是 *CBR1* 基因表达的必需因素。

关键词：猪；*CBR1*基因；启动子；核转录因子kappa B（NF-κB）；子宫内膜