Theriogenology 85 (2016) 1476–1482

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

BDNF-induced expansion of cumulus-oocyte complexes in pigs was mediated by microRNA-205

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ARTICLE INFO

Article history:
Received 20 August 2015
Received in revised form 11 December 2015
Accepted 5 January 2016

Keywords:
BDNF
miR-205
Ptx3
COC
Maturation

ABSTRACT

The neurotrophin family of proteins is required for the survival and differentiation of the nervous system and is important to the development of reproductive tissues. The objectives of the present study were to detect the presence of the brain-derived neurotrophic factor (BDNF) and its tyrosine kinase receptor protein in cumulus-oocyte complexes in pigs and to explore the role of microRNAs in the BDNF-induced *in vitro* maturation of oocytes. We demonstrate that both BDNF and tyrosine kinase receptor protein are expressed in porcine cumulus oocyte complexes. BDNF supplementation promotes the *in vitro* maturation of porcine oocytes. MiRNA-205 is downregulated during the BDNF-induced maturation of oocytes. The overexpression of miRNA-205 in granulosa cells and reporter gene assay shows that the marker gene ptx3 for cumulus expansion is the putative target gene of miR-205. Our data provide evidence that the BDNF-induced maturation of oocytes in pigs may be mediated by miR-205 through the regulation of potential target gene, ptx3.

1. Introduction

The successful *in vitro* maturation of oocytes is an essential step in the research on transgenic, cloning, and animal breeding programs. However, the developmental competence of oocytes that undergo *in vitro* maturation is less than that of oocytes that undergo *in vivo* maturation because of an unclear regulating mechanism [1]. The mechanism of oocyte maturation must be explored to facilitate the optimization of the *in vitro* maturation system. In the process of *in vivo* maturation, the complex signaling networks within follicles are critical to increase the developmental competence of oocytes and to expand cumulus cells [2]. Aside from the signaling from the LH surge during ovulation and oocyte maturation [3], autocrine and paracrine stimuli within the cumulus-oocyte complexes (COCs) are also involved in the oocyte maturation [4]. A growing body of evidence has indicated that the brain-derived neurotrophic factor (BDNF), a member of neurotrophins, is present in oocytes and somatic cells in the ovary [5]. The binding of the said factor to a high-affinity transmembrane receptor tyrosine kinase receptor protein (TrkB) is required for cell survival and growth. The direct roles of BDNF in the ovarian development have been explored using TrkB gene knockout mouse models [6]. A disruption in the BDNF/TrkB signaling also disrupts the follicular development [6]. Furthermore, *in vitro* experiments have shown that BDNF can enhance oocyte maturation and embryo development in mice, cattle, and pigs [7,8].

Some noncoding RNAs, such as microRNAs (miRNA), have been recently detected in the reproductive tissues and have been found to be essential to ovarian development, oocyte maturation, and embryo development [9]. Some studies have suggested that BDNF exerts a beneficial effect

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0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.01.004

on the central nervous system by regulating the expression of miRNA, such as miR-132 [10]. The aims of the present study were to detect the presence of the BDNF and its TrkB in COCs in pigs and to explore the role of microRNAs in the BDNF-induced *in vitro* maturation of oocytes.

2. Materials and methods

2.1. Chemical, media, and culture conditions

All the drugs used in the present study were purchased from Boster (Wuhan, China), unless stated otherwise. The medium TCM-199, which was supplemented with 10% porcine follicular fluid, 0.57 mmol/L of cysteine, 3.05 mmol/L of glucose, 0.1% polyvinyl alcohol, and 0.91 mmol/L of sodium pyruvate, was used for maturation. The BDNF was purchased from Cell Signaling Technology Inc. (Beverly, MA, USA) and was diluted with water.

2.2. Oocyte collection and maturation

2.2.1. Ovary and oocyte collection

Porcine ovaries were collected from prepubertal gilts at a local slaughterhouse and were transported to the laboratory in a 0.9% NaCl solution that was supplemented with antibiotics for 4 hours. The COCs were aspirated from the middle size of follicles (3–5 mm).

2.2.2. In vitro maturation

The *in vitro* maturation of oocytes was conducted as described previously [11]. The COCs (i.e., 10 COCs/100 μL drop) were matured in an IVM medium (i.e., TCM-199 supplemented with 10% porcine follicular fluid + 0.57 mmol/L of cysteine + 3.05 mmol/L glucose + 0.1% polyvinyl alcohol + 0.91 mmol/L sodium pyruvate). After 24/48 hours of culture at 39 °C and under 5% CO_2 condition, the COCs were collected for the subsequent experiments.

2.3. RNA preparation, Reverse transcription-polymerase chain reaction (RT-PCR), and Quantitative RT-PCR analysis

The total RNAs of COCs, granulosa cells (GCs), and oocytes were extracted using the TRIzol reagent (Invitrogen Co., Foster, CA, USA) according to the manufacturer's instructions. One microgram of DNase-treated total RNA was reverse transcribed to a first-strand cDNA using SuperScript III Reverse Transcriptase (Invitrogen Co., Foster, CA, USA) according to the manufacturer's instructions. The RT-PCR was performed by denaturation at 95 °C for 3 minutes followed by 30 cycles of 95 °C for 30 seconds, 58 °C for 30 seconds, and 72 °C for 40 seconds, and a final extension at 72 °C for 10 minutes. The expression levels of the genes were detected through real-time PCR under ABI PRISM 7000, following the described instructions. The specific primers used for amplifying genes in the present study are summarized in Table 1.

2.4. Immunofluorescence

The COCs were aspirated from the middle size of follicles and were then rinsed three times with PBS. The COCs were fixed with precooled absolute methanol for 10 minutes and treated with 0.1% Triton X-100 (Sigma Co., CA, USA) for 5 minutes at room temperature. The COCs were then blocked with normal goat serum (10%) in PBS at room temperature for 30 minutes and incubated with BDNF and TrkB antibodies (1:300 dilution; Boster Co., Wuhan, China) at 4 °C overnight. After three washes with PBS, the slides were exposed to goat anti-rabbit IgG conjugated with fluorescein isothiocyanate (FITC, 1:100 dilution; Boster Co., Wuhan, China) for 60 minutes at room temperature and were then washed with PBS. The negative controls were obtained by incubating the slides with BSA diluted with PBS instead of the primary antibody. The slides covered with 90% glycerol were finally examined under a fluorescence microscope (Olympus Co., Tokyo, Japan).

Table 1
List of primers used for polymerase chain reaction, reverse transcription, and plasmid construction.

Primer name	Sequence 5′-3′
BDNF-F	GTTTCCCTCTGGTCATGGAA
BDNF-R	GCTGGCGGTTCATAAGGATA
TrkB-F	CCAACCTCAGACCACCAC
TrkB-R	TTTCTCATCCTTCCCATAC
Ptx3-F	TGCCAGCAGGTTGTGAAA
Ptx3-R	AATGCAGGCACTGAAAGC
miR-125-F	CGTCCCTGAGACCCT
miR-125-R	GTGCAGGGTCCGAGGT
miR-574-F	CACGCUCAUGCACACA
miR-574-R	GTGCAGGGTCCGAGGT
miR-29a-F	CGCTAGCACCATCTGAAAT
miR-29a-R	GTGCAGGGTCCGAGGT
miR-205-F	CGTCCAACATTCCACCG
miR-205-R	GTGCAGGGTCCGAGGT
U6-F	CTCGCTTCGGCAGCACA
U6-R	AACGCTTCACGAATTTGCGT
Reverse transcription primers	
miR-125	GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACCACAGG
miR-574	GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACTGTGGG
miR-29a	GTCGTATCCAGTGCAGGGTCCGAGGTA TTCGCACTGGATACGACTAACCG
miR-205	GTCGTATCCAGTGCAGGGTCCGAGGT ATTCGCACTGGATACGACCAGACTC
Plasmid construction primers	
Ptx3-F	AGTGTTGTGAAACTCTACTCG
Ptx3-R	GAGCAATGAACAACATGTG
miR-205-F	CAGAGGGTGTGAGGACTCGGAT
miR-205-R	TTGTAACCCAAAGGGGCGG

2.5. Western blotting

Cells were lysed with a lysis buffer (i.e., 10 mM of Tris-HCl, pH 7.4, 50 mM of sodium chloride, 30 mM of sodium pyrophosphate, 50 mM of sodium fluoride, 100 mM of sodium orthovanadate, 2 mM of iodoacetic acid, 5 mM of $ZnCl_2$, 1 mM of phenylmethylsulfonyl fluoride, and 0.5% Triton-X 100) for protein isolation (Beyotime Bio, China). A total of 20 μg of normalized proteins from each sample were separated by 10% SDS-PAGE and subsequently transferred onto polyvinylidene fluoride membranes (Millipore Co., USA) at 80 V for 1.5 hours (Bio-Rad wet transfer system). After 2 hours of blocking with a mixture of Tris-Buffered Saline and Tween 20 (TBST) containing 5%