

The role of brain-derived neurotrophic factor in mouse oocyte maturation *in vitro* involves activation of protein kinase B

L. Zhang^a, Y. Liang^b, Y. Liu^a, C.-L. Xiong^{a,*}

^aFamily Planning Research Institute and Centre for Reproductive Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, PR China

^bDepartment of Social Medicine, Public Health School, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, PR China

Received 13 July 2009; received in revised form 24 December 2009; accepted 9 January 2010

Abstract

Brain-derived neurotrophic factor (BDNF) can promote developmental competence in mammalian oocytes during *in vitro* maturation, but the signal transduction pathways are not clear. In this study, we investigated (using western blots) the effects of BDNF on the phosphorylation of protein kinase B (PKB) and mitogen-activated protein kinase (MAPK) in mouse oocytes and cumulus cells cultured *in vitro*. Treatment with BDNF enhanced phosphorylation of PKB in oocytes at 2 h ($P = 0.0006$) and 3 h ($P < 0.0001$) of *in vitro* maturation, compared with control oocytes. However, the pan-specific tyrosine kinase (Trk) inhibitor K252a together with BDNF completely inhibited phosphorylation of PKB in the oocytes. Furthermore, BDNF increased phosphorylation of MAPK in oocytes at 16 h of *in vitro* maturation ($P = 0.0041$), but K252a together with BDNF did not reduce phosphorylation of MAPK in the oocytes. For cumulus cells, BDNF significantly prolonged the phosphorylation of PKB and MAPK and increased the total amounts of PKB and MAPK proteins after 16 h of *in vitro* maturation. However, BDNF did not affect apoptosis of the cumulus cells during oocyte maturation *in vitro*. In conclusion, the PKB pathway is likely to be one signaling cascade activated by BDNF in combination with the TrkB receptor, whereas the MAPK pathway is not involved. These findings may have relevance for BDNF-induced promotion of developmental capacity of *in vitro*-matured oocytes.

© 2010 Elsevier Inc. All rights reserved.

Keywords: Brain-derived neurotrophic factor; Oocytes; *In vitro* maturation; Protein kinase B; Mitogen-activated protein kinase

1. Introduction

Neurotrophins comprise a family of soluble polypeptide growth factors widely recognized for their roles in the mammalian nervous system. Although these factors are expressed widely in the central nervous system and are important for neuronal survival and

differentiation [1], they also play important roles in non-neuronal tissues [2]. Brain-derived neurotrophic factor (BDNF) is one member of the family of neurotrophins. It exists in mouse ovarian follicles and it is secreted by granulosa and cumulus cells [3,4]. The high-affinity tyrosine kinase type B (TrkB) receptor for BDNF was expressed exclusively in oocytes, whereas the pan-neurotrophin low-affinity receptor p75 (p75NTR) is detected in granulosa cells, oocytes, and theca cells in mouse preovulatory follicles [4]. Based on gene knockout experiments, BDNF was essential for folliculogenesis and early follicle development in the

* Corresponding author. Tel.: +86 27 83692651; fax: +86 27 83692651.

E-mail address: clxiongcn@yahoo.com.cn (C.L. Xiong).

mouse [5–7]. Brain-derived neurotrophic factor and its receptors are also found in bovine, porcine, and human ovaries [3,8–10]. Moreover, the presence of BDNF during oocyte maturation *in vitro* promoted preimplantation development of mouse, bovine, and porcine embryos [4,8,9]. Based on these results, BDNF may act as a paracrine and/or autocrine growth factor and regulate follicle and oocyte development. However, little is known about the detailed downstream molecular events after BDNF acts on its receptors in the follicle.

In neurons, Trks and their substrates can activate three main signaling cascades [11]: (1) the small GTP-binding protein Ras and mitogen-activated protein kinase kinase (MAPKK)/MAP kinase pathway; (2) the phospholipid kinase PI-3 kinase (PI3K) and protein kinase B (PKB); and (3) the phospholipase C- γ (PLC- γ) pathway, which results in the formation of the second messengers diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP₃) and calcium release from intracellular stores. All of these pathways may be relevant to follicular and oocyte maturation.

Mitogen-activated protein kinase is involved in the regulation of microtubule organization, meiotic spindle assembly, and maintenance of metaphase II arrest in the oocyte [12]. The PI3K-PKB pathway is involved in resumption of meiosis in *Xenopus*, mouse, rat, bovine, and porcine oocytes [13–18]. Moreover, phosphorylated PKB is involved in the completion of meiosis at fertilization, including the second polar body emission and the organization of microtubules [15]. We found that BDNF improved spindle configuration and location, which may account for promotion of ooplasmic maturation by BDNF (unpublished data). In the present study, the effects of BDNF on PKB and MAPK phosphorylation levels in oocytes and cumulus cells were investigated to clarify the BDNF signal transduction pathways acting during oocyte maturation.

2. Materials and methods

2.1. Animals

Kunming mice were from the Animal Experiment Center of Tongji Medical College of Huazhong University of Science and Technology. Female mice, 4- to 5-weeks old, were used. They were kept in a room with 14 h/10 h light-dark cycles (dark starting at 20:00) and food and water were provided *ad libitum*. All protocols and experimental procedures for the use of animals in this study were performed in accordance

with the NIH Guiding Principles in the Care and Use of Animals.

2.2. Chemicals

All chemicals were of the purest analytical grade and were purchased from Amresco (Solon, OH, USA), unless otherwise indicated.

2.3. Oocyte collection, culture and use in experiments

Mice were given 10 IU of pregnant mare serum gonadotropin (Hangzhou Animal Medicine Factory, Hangzhou, P. R. China) ip, and 48 h later, cumulus-oocyte complexes (COCs) were obtained by puncturing the largest follicles in HEPES-buffered α -minimum essential medium (α -MEM; HyClone Biochemical Products Co., Ltd., Beijing, P. R. China), supplemented with Earle's salts, 50 μ g/mL streptomycin sulfate, 75 μ g/mL penicillin G, 18 mM HEPES, and 4 mM hypoxanthine. The COCs with more than three layers of cumulus cells were chosen and washed. They were then transferred to 25 μ L drops of maturation medium (10 COCs per drop), covered with mineral oil (Sigma-Aldrich Co., St. Louis, MO, USA), and cultured at 37 °C in a humidified atmosphere of 5% CO₂ in air.

According to the *in vitro* maturation (IVM) medium used, COCs were divided into three groups:

- (1) Stock IVM medium (the control group), based on α -MEM supplemented with Earle's salts, 50 μ g/mL streptomycin sulfate, 75 μ g/mL penicillin G, 0.23 mM sodium pyruvate, and 5% (v/v) fetal bovine serum (FBS; HyClone).
- (2) Stock IVM medium supplemented with 5 ng/mL BDNF (Promega, Madison, WI, USA; BDNF-treated group). Brain-derived neurotrophic factor was indirectly dissolved in α -MEM at 100 ng/mL and stored in small volumes at -20 °C. We used 5 ng/mL BDNF, in accordance with a previous report [4] and our own preliminary experiment.
- (3) Stock IVM medium supplemented with 5 ng/mL BDNF and 100 nM K252a (K252a-treated group). The K252a (Biosource International, Inc., Camarillo, CA, USA) is a pan-specific Trk inhibitor; it was dissolved in dimethyl sulfoxide (DMSO; Sigma) and diluted in the stock IVM medium at 100 nM, as described [4]. The final concentration of DMSO in the stock IVM medium was 0.005%, which had no significant effect on the experiments.

2.4. Western blot analysis

The COCs used for western blot were collected at 0, 1, 2, 3, 6, and 16 h of culture *in vitro*, and washed in protein-free medium (HEPES-buffered α -MEM supplemented with 10 mg/mL polyvinyl alcohol; Sigma). After cumulus cells had been removed mechanically by repeated pipetting with small-bore Pasteur pipettes, oocytes and the cumulus cells were placed separately in Eppendorf tubes and stored frozen at -70°C .

Western blot was carried out according to the manufacturer's instructions, with minor modifications. Briefly, oocytes were lysed in a NuPAGE LDS sample buffer (Invitrogen-GIBCO, Carlsbad, CA, USA), containing a reducing agent, denatured at 70°C for 10 min, cooled on ice for 5 min, and centrifuged at $10,000 \times g$ and 4°C for 5 min. Samples were then loaded onto Novex pre-cast gels (Invitrogen). Cumulus cells from IVM cultures for 0, 1, 2, 6, and 16 h were lysed in radioimmunoprecipitation buffer (Cell Signaling Technology, Beverly, MA, USA) and quantified using Pierce BCA protein assay kits (Pierce, Rockford, IL, USA). The prepared samples were aliquoted and stored at -70°C for further use. Aliquots containing $8 \mu\text{g}$ of sample protein for cumulus cells were mixed into the NuPAGE LDS sample buffer; the subsequent protocol was the same as that for oocytes. All gels for western blotting included pre-stained protein molecular weight markers (Invitrogen). Proteins in the gels were transferred onto Invitrolon polyvinylidene difluoride membranes (Invitrogen).

To investigate the effects of BDNF on PKB and MAPK phosphorylation status, mouse anti-phospho-PKB-Ser473 (587F11) monoclonal antibody (Cell Signaling Technology), and rabbit anti-phospho-MAPK1/2 (Thr202/Tyr204) polyclonal antibody (CHEMICON International Inc., Germany) were used. Western Breeze kits (Invitrogen) were used after immunodetection of western blots, in accordance with the manufacturer's protocols. Chemiluminescence intensities of the bands in membranes were visualized on X-Omat LS films (Kodak, New Haven, CT, USA). Then, the previous phosphorylation antibody was stripped from the membrane in a stripping buffer (Beyotime, Haimen, P. R. China) for 5 min at 37°C under permanent agitation. After being blocked, the membrane was incubated with a rabbit polyclonal anti-PKB antibody (Cell Signaling Technology for total PKB protein detection) or a rabbit anti-MAPK2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). In the above cases, the primary antibody dilution was 1:1000.

As a loading control and internal standard, membranes for cumulus cells protein were reprobated with a rabbit antibody against β -actin (Abcam, Cambridge, UK). For this, after chemiluminescence detection, the previous antibody was stripped from the membrane, using the stripping buffer as described above. After being blocked, the membrane was incubated with anti- β -actin antibody diluted 1:2000. The following steps were the same as described above.

In all cases, the intensity of each band was measured using Kodak Digital Science 1D image analysis software (Kodak, Version 2.0). For quantification, the ratio of the intensity of the protein concerned (p-PKB or p-MAPK) is presented in relation to the measured levels of PKB and MAPK in oocytes and cumulus cells.

2.5. Assessment of apoptosis

Cumulus cells that had undergone apoptosis were identified with the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) cell apoptosis detection kit (Beyotime). After 16 h of culture, COCs designated to be analyzed for apoptosis were treated in 80 IU/mL hyaluronidase for 30 s and then separated by repeated pipetting. The cumulus cells were collected and centrifuged at $300 \times g$ for 10 min. After being washed with PBS (pH 7.4), cumulus cells were fixed with 4% paraformaldehyde for 60 min. They were then washed with PBS, centrifuged, and pipetted onto polylysine coated glass slides. Cumulus cells were permeabilized with 0.1% Triton X-100 for fluorescein isothiocyanate (FITC) end-labeling of the fragmented DNA of the apoptotic cells using TUNEL cell apoptosis detection kits. The cell nuclei were stained with $2 \mu\text{g}/\text{mL}$ Hoechst 33258 (Sigma). The FITC-labeled TUNEL-positive cells were imaged under an inverted laser scanning confocal microscope (Olympus, Tokyo, Japan; FV500-IX71) at a magnification of $\times 400$, using 488 nm wavelength light for excitation and 530 nm for emission. The nuclei of cumulus cells were imaged using 350 nm stimulation and 460 nm emission. Approximately 200 cumulus cells were observed on each slide and the percentage of cells with apoptotic nuclei was calculated as the apoptotic index.

2.6. Statistical analysis

Data are presented as the mean \pm SD. For oocytes and cumulus cells, the effect of BDNF on the phosphorylation of PKB and MAPK were evaluated by GLM repeated measures ANOVA. Moreover, multiple comparisons were performed for the intensity

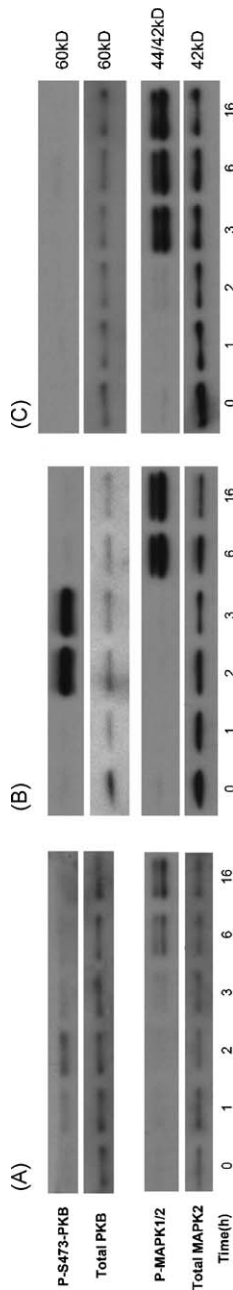


Fig. 1. Kinetics of the phosphorylation of PKB and MAPK in oocytes cultured in the stock maturation medium (A) supplemented with BDNF (B) or with BDNF and K252a (C). The blot was first probed with p-Ser473-PKB and p-MAPK1/2 antibody, then stripped and reprobed with total PKB and MAPK2 antibodies. Each lane of the SDS-PAGE gel contained 50 oocytes. This represented four similar experiments.

ratios for p-MAPK/MAPK in the oocytes among the control, BDNF-treated, and K252a-treated groups. Apoptotic indices of cumulus cells were compared by one-way ANOVA. Statistical analyses were conducted using the Statistical Package for Social Sciences program, Version 11.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was assumed at $P < 0.05$.

3. Results

3.1. Effects of BDNF on PKB phosphorylation in oocytes and cumulus cells

We investigated the time course of PKB phosphorylation in oocytes and cumulus cells during IVM. Brain-derived neurotrophic factor enhanced phosphorylation of PKB compared with the control oocytes ($P < 0.0001$; Fig. 1; Table 1). There was an interaction between groups (control and BDNF-treated) and culture time (IVM 2 h and 3 h) on the phosphorylation of PKB in the oocytes ($P = 0.0003$). The phosphorylation of PKB in the control oocytes was detected at IVM 1 h, reached a maximum at 2 h, and was greatly reduced at 3 h (Fig. 1A). For BDNF-treated oocytes, phosphorylation of PKB increased rapidly at 2 h and peaked at 3 h of culture (Fig. 1B; $P = 0.0006$ for IVM 2 h and $P < 0.0001$ for IVM 3 h compared to the control oocytes at the same time). No phosphorylation of PKB was detected in K252a-treated oocytes (Fig. 1C). The amount of total PKB protein in oocytes did not change at any time during IVM.

Brain-derived neurotrophic factor treatment prolonged the activation time of PKB in cumulus cells compared with the control (Fig. 2; Table 2; $P = 0.0064$). There was an effect of culture time ($P < 0.0001$) and an interaction between groups and culture time on the phosphorylation of PKB in the cumulus cells ($P = 0.018$). Phosphorylated PKB levels in the cumulus cells in both groups gradually decreased as culture time increased. Phosphorylation of PKB in the cumulus cells between BDNF-treated and the control groups was not different at the beginning of culture and IVM 1 h. However, phosphorylation of PKB in the control cumulus cells reduced after 2 h (Fig. 2A). The phosphorylation level of PKB in the BDNF-treated cumulus cells was higher than that in the control at 2 and 6 h of IVM (Table 2; $P = 0.021$ for IVM 2 h and $P = 0.0018$ for IVM 6 h). Moreover, the amount of total PKB protein in the control cumulus cells decreased at IVM 16 h whereas that in the BDNF-treated cumulus cells remained unchanged at all time points (Fig. 2).

Table 1

Mean \pm SD quantitative analysis of western blot of the phosphorylation of PKB (p-PKB/PKB) within murine oocytes matured *in vitro*.

Group	IVM 2 h	IVM 3 h	P value		
			Time	Group	Time by group interaction
Control	1.62 \pm 0.03 ^a	0.76 \pm 0.06 ^c	0.34	< 0.0001	0.0003
BDNF	2.55 \pm 0.25 ^b	3.22 \pm 0.11 ^d			

This experiment was repeated four times. Cumulus-oocyte complexes were cultured in the stock *in vitro* maturation medium supplemented with or without 5 ng/mL BDNF (control).

^{a-d}Within each column, means without a common superscript differed ($P < 0.05$).

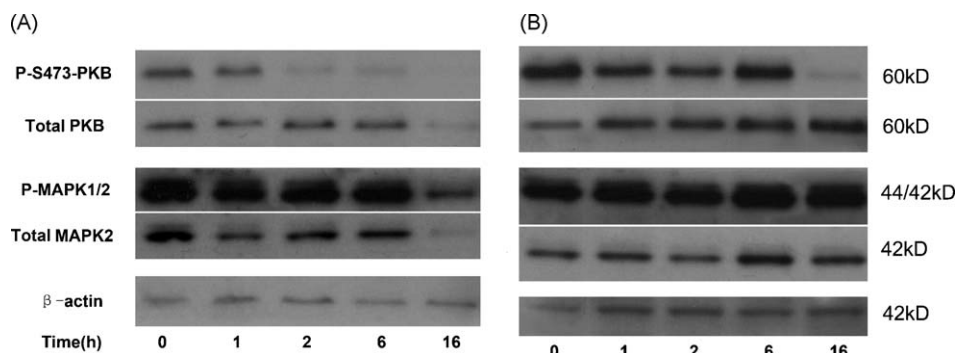


Fig. 2. Kinetics of the phosphorylation levels of PKB and MAPK in cumulus cells cultured in the stock maturation medium (A) supplemented with BDNF (B). 8 μ g protein was loaded per lane for cumulus cells. This represented three similar experiments.

Table 2

Mean \pm SD quantitative analysis of western blot of the phosphorylation of PKB (p-PKB/PKB) within murine cumulus cells during oocyte maturation *in vitro*.

Group	0	IVM 1 h	IVM 2 h	IVM 6 h	P value		
					Time	Group	Time by group interaction
Control	1.88 \pm 0.16	1.44 \pm 0.07	0.73 \pm 0.12 ^a	0.59 \pm 0.10 ^c	< 0.0001	0.0064	0.018
BDNF	2.07 \pm 0.28	1.24 \pm 0.10	1.11 \pm 0.13 ^b	1.01 \pm 0.03 ^d			

This experiment was repeated three times.

Cumulus-oocyte complexes were cultured in the stock *in vitro* maturation medium supplemented with or without 5 ng/mL BDNF (control).

^{a-d}Within each column, means without a common superscript differed ($P < 0.05$).

3.2. Effects of BDNF on MAPK phosphorylation in oocytes and cumulus cells

Treatment with BDNF enhanced the phosphorylation of MAPK compared with the control oocytes (Fig. 1; Table 3; $P < 0.0001$). Phosphorylated MAPK was detected at IVM 3 h in the control oocytes and was greatly elevated at 6 to 16 h (Fig. 1A). The phosphorylation of MAPK was enhanced at IVM 16 h in BDNF-treated oocytes compared with the control ($P = 0.0041$; Table 3; Fig. 1B). However, it was obviously increased at IVM 3 h and maintained to 16 h when K252a was added into the stock IVM medium supplemented with BDNF (K252a-treated group; Fig. 1C, Table 3).

Treatment with BDNF prolonged MAPK phosphorylation in cumulus cells (Fig. 2; Table 4; $P = 0.0018$). In control cumulus cells, MAPK phosphorylation peaked at 0 to 6 h and was reduced at 16 h (Fig. 2A), whereas in the BDNF-treated cumulus cells it remained unchanged until 16 h of culture (Fig. 2B). In addition, the amount of total MAPK protein in the control cumulus cells decreased at IVM 16 h, whereas in the BDNF-treated cumulus cells it remained unchanged (Fig. 2).

3.3. Effects of BDNF on cumulus cell apoptosis

The apoptotic indices of cumulus cells in the control, BDNF-treated and K252a-treated groups were

Table 3

Mean \pm SD quantitative analysis of western blot of the phosphorylation of MAPK (p-MAPK/MAPK) within murine oocytes matured *in vitro*.

Group	IVM 3 h	IVM 6 h	IVM 16 h	P value		
				Time	Group	Time by group interaction
Control	0 ^a	1.85 \pm 0.12	1.92 \pm 0.07 ^c	< 0.0001	< 0.0001	< 0.0001
BDNF	0 ^a	2.22 \pm 0.26	2.51 \pm 0.19 ^d			
BDNF + K252a	2.01 \pm 0.20 ^b	2.14 \pm 0.22	2.33 \pm 0.26 ^d			

This experiment was repeated four times.

Cumulus-oocyte complexes were cultured in the stock *in vitro* maturation medium (control), supplemented with 5 ng/mL BDNF, or with 5 ng/mL BDNF plus 100 nM K252a.

^{a-d}Within each column, means without a common superscript differed ($P < 0.05$).

Table 4

Mean \pm SD quantitative analysis of western blot of the phosphorylation of MAPK (p-MAPK/MAPK) within murine cumulus cells during oocyte maturation *in vitro*.

Group	0	IVM 1 h	IVM 2 h	IVM 6 h	IVM 16 h	P value		
						Time	Group	Time by group interaction
Control	1.95 \pm 0.07	1.78 \pm 0.18	1.74 \pm 0.19	1.85 \pm 0.06	0 ^a	<0.0001	0.0018	<0.0001
BDNF	1.92 \pm 0.12	1.88 \pm 0.06	2.02 \pm 0.17	1.94 \pm 0.11	1.91 \pm 0.07 ^b			

This experiment was repeated three times and data were shown as the cumulus-oocyte complexes were cultured in the stock IVM medium (control), supplemented with 5 ng/mL BDNF.

^{a-d}Within each column, means without a common superscript differed ($P < 0.05$).

1.15 \pm 0.11, 0.88 \pm 0.29, and 1.10 \pm 0.23%, respectively ($P = 0.26$).

4. Discussion

Addition of BDNF to the IVM medium enhanced phosphorylation of PKB and prolonged the activation time of PKB in the oocytes. Moreover, PKB phosphorylation in the oocytes was almost completely inhibited by the TrkB inhibitor K252a. We inferred that PKB was probably phosphorylated by BDNF via a TrkB type receptor on the surface of the oocyte. In addition, PKB phosphorylation in the oocyte caused by endogenous factors was also inhibited by K252a, perhaps because K252a is a nonspecific inhibitor of the Trk family.

Protein kinase B is phosphorylated at two residues, Thr³⁰⁸ and Ser⁴⁷³ [19]. However, the separate phosphorylation of either of these is sufficient for PKB activation [20,21]. Furthermore, pSer⁴⁷³ PKB is the major form in the PKB pool, whereas pThr³⁰⁸ PKB comprises only a small subset [22]. Therefore, only pSer⁴⁷³ PKB was detected in our experiments. Although Thr³⁰⁸-phosphorylated PKB was present in pericentriolar material, localization of Ser⁴⁷³-phosphorylated PKB was similar to that of microtubules in metaphase oocytes [15]. These forms are individually involved in fertilization to complete meiosis, including emission of

the second polar body and organization of microtubules [15]. We had already found that BDNF promoted cytoplasmic maturation in immature oocytes, which supported early mouse embryo development and improved meiotic spindle configuration and location (unpublished data). The width and area of the meiotic spindle in oocytes cultured in IVM medium supplemented with BDNF were significantly smaller than those cultured without BDNF. Moreover, more oocytes had spindles positioned near the oolemma in the BDNF-treated group than in the control. Normal spindle morphology is critical to maintain normal meiosis in the oocyte; furthermore, various characteristics of the spindle, including its presence, location, and length, are associated with oocyte quality [23]. Therefore, an increase in PKB phosphorylation caused by BDNF may be one reason that BDNF promotes oocyte cytoplasmic maturation.

In this study, BDNF significantly increased MAPK phosphorylation in oocytes. However, this activity was unaffected when K252a was added into the maturation media supplemented with BDNF. Brain-derived neurotrophic factor and TrkB activate MAPK via Ras, but MAPK is activated by Mos (a kind of MAPKKK) in oocytes [12]. Therefore, the stimulating effect of BDNF on the phosphorylation of MAPK was unlikely to be mediated by the TrkB receptor. Increases in MAPK

phosphorylation within the BDNF-treated oocytes may be due to BDNF-induced phosphorylation of the cAMP responsive element-binding protein (CREB) and activation of CREB-dependent transcription [24].

The role of K252a is very complex, although it is often used as an inhibitor of the Trk family. It also inhibits protein kinase A, myosin light chain kinase, protein kinase C, Ca²⁺/calmodulin-dependent protein kinases, MAPK, and numerous other kinases. However, K252a may indirectly activate MAPK [25], which could be why MAPK phosphorylation peaked early in the K252a-treated oocytes in the present study. In our previous experiments, K252a alone in the IVM medium did not influence germinal vesicle breakdown, emission of the first polar body or fertilization *in vitro* in oocytes matured *in vitro* (unpublished data). Concomitant treatment with BDNF and K252a significantly suppressed the stimulatory effect of BDNF on blastocyst formation (unpublished data). Furthermore, TrkB was expressed exclusively in oocytes in mouse preovulatory follicles [4]. Therefore, we chose K252a to study the signal pathway of BDNF during oocyte IVM.

Brain-derived neurotrophic factor significantly prolonged PKB and MAPK phosphorylation in cumulus cells. Moreover, BDNF increased the total amount of PKB and MAPK proteins within the cumulus cells compared with controls at 16 h of IVM. The increase in MAPK phosphorylation in the cumulus cells at IVM 16 h may be related to the increase in the amount of total MAPK protein in the BDNF-treated group. Although both PKB and MAPK can promote cell survival, in the present study, BDNF did not significantly reduce apoptosis of cumulus cells. In mouse preovulatory follicles, cumulus cells were found to only express p75NTR [4]. Therefore, the increased phosphorylation of PKB and MAPK caused by BDNF may have been caused via the p75NTR receptor.

In conclusion, we inferred that the PKB pathway was likely to be one signaling cascade activated by BDNF, in combination with the TrkB receptor in the oocyte, whereas the MAPK pathway was not. Brain-derived neurotrophic factor promoted the phosphorylation of PKB and MAPK in oocytes matured *in vitro*. Thus, BDNF might improve the capacity of the IVM oocyte to develop to preimplantation embryos.

Acknowledgments

This work was partially supported by grants from National ‘Ten times Five Years’ Key Technologies R&D Program, P. R. China (No. 2004BA720A33-01)

and by funds from Tongji Medical College of Huazhong University of Science and Technology.

References

- [1] Tessarollo L. Pleiotropic functions of neurotrophins in development. *Cytokine Growth Factor Rev* 1998;9:125–37.
- [2] Yamamoto M, Sobue G, Yamamoto K, Terao S, Mitsuma T. Expression of mRNAs for neurotrophic factors (NGF, BDNF, NT-3, and GDNF) and their receptors (p75^{NGFR}, TrkA, TrkB, and TrkC) in the adult human peripheral nervous system and nonneuronal tissues. *Neurochem Res* 1996;21:929–38.
- [3] Seifer DB, Feng B, Shelden RM, Chen S, Dreyfus CF. Brain-derived neurotrophic factor: a novel human ovarian follicular protein. *J Clin Endocrinol Metab* 2002;87:655–9.
- [4] Kawamura K, Kawamura N, Mulders SM, Gelpke MD, Hsueh AJ. Ovarian brain-derived neurotrophic factor (BDNF) promotes the development of oocytes into preimplantation embryos. *PNAS* 2005;102:9206–11.
- [5] Ofeda SR, Romero C, Tapia C, Dissen GA. Neurotrophic and cell-cell dependent control of early follicular development. *Mol Cell Endocrinol* 2000;163:67–71.
- [6] Paredes A, Romero C, Dissen GA, DeChiara TM, Reichardt L, Cornea A, et al. TrkB receptors are required for follicular growth and oocyte survival in the mammalian ovary. *Dev Biol* 2004; 267:430–49.
- [7] Kerr B, Garcia-Rudaz C, Dorfman M, Paredes A, Ojeda SR. NTRK1 and NTRK2 receptors facilitate follicle assembly and early follicular development in the mouse ovary. *Reprod* 2009; 138:131–40.
- [8] Martins da Silva SJ, Gardner JO, Taylor JE, Springbett A, De Sousa PA, Anderson RA. Brain-derived neurotrophic factor promotes bovine oocyte cytoplasmic competence for embryo development. *Reprod* 2005;129:423–34.
- [9] Lee E, Jeong YI, Park SM, Lee JY, Kim JH, Park SW, et al. Beneficial effects of brain-derived neurotrophic factor on *in vitro* maturation of porcine oocytes. *Reprod* 2007;134:405–14.
- [10] Seifer DB, Feng B, Shelden RM. Immunocytochemical evidence for the presence and location of the neurotrophin-Trk receptor family in adult human preovulatory ovarian follicles. *Am J Obstet Gynecol* 2006;194:1129–34.
- [11] Rose CR, Blum R, Kafitz KW, Kovalchuk Y, Konnerth A. From modulator to mediator: rapid effects of BDNF on ion channels. *BioEssays* 2004;26:1185–94.
- [12] Fan HY, Sun QY. Involvement of mitogen-activated protein kinase cascade during oocyte maturation and fertilization in mammals. *Biol Reprod* 2004;70:535–47.
- [13] Andersen CB, Sakaue H, Nedachi T, Kovacina KS, Clayberger C, Conti M, Roth RA. Protein kinase B/Akt is essential for the insulin-but not progesterone-stimulated resumption of meiosis in *Xenopus* oocytes. *BioChem J* 2003;369:227–38.
- [14] Han SJ, Vaccari S, Nedachi T, Andersen CB, Kovacina KS, Roth RA, Conti M. Protein kinase B/Akt phosphorylation of PDE3A and its role in mammalian oocyte maturation. *EMBO J* 2006;25: 5716–25.
- [15] Hoshino Y, Sato E. Protein kinase B (PKB/Akt) is required for the completion of meiosis in mouse oocytes. *Dev Biol* 2008;314: 215–23.
- [16] Reddy P, Shen L, Ren C, Boman K, Lundin E, Ottander U, et al. Activation of Akt (PKB) and suppression of FKHL1 in mouse

- and rat oocytes by stem cell factor during follicular activation and development. *Dev Biol* 2005;281:160–70.
- [17] Tomek W, Smiljakovic T. Activation of Akt (protein kinase B) stimulates metaphase I to metaphase II transition in bovine oocytes. *Reprod* 2005;130:423–30.
- [18] Shimada M, Ito J, Yamashita Y, Okazaki T, Isobe N. Phosphatidylinositol 3-kinase in cumulus cells is responsible for both suppression of spontaneous maturation and induction of gonadotropin-stimulated maturation of porcine oocytes. *J Endocrinol* 2003;179:25–34.
- [19] Alessi DR, Cohen P. Mechanism of activation and function of protein kinase B. *Curr Opin Genet Dev* 1998;8:55–62.
- [20] Lengyel F, Vertes Z, Kovacs KA, Kornyei JL, Sumegi B, Vertes M. Expression and activation of Akt/protein kinase B in sexually immature and mature rat uterus. *J Steroid Biochem Mol Biol* 2004;91:285–8.
- [21] Viniegra JG, Martinez N, Modirassari P, Losa JH, Parada Cobo C, Lobo VJ, et al. Full activation of PKB/Akt in response to insulin or ionizing radiation is mediated through ATM. *J Biol Chem* 2005;280:4029–36.
- [22] Kalous J, Solc P, Baran† V, Kubelka M, Schultz‡ MK, Motlik J. PKB/AKT is involved in resumption of meiosis in mouse oocytes. *Biol Cell* 2006;98:111–23.
- [23] Wang Q, Sun QY. Evaluation of oocyte quality: morphological, cellular and molecular predictors. *Reprod Fertil Dev* 2007;19:1–12.
- [24] Huang EJ, Reichardt LF. Trk receptors: roles in neuronal signal transduction. *Annu Rev Biochem* 2003;72:609–42.
- [25] Roux PP, Dorval G, Boudreau M, Angers-Loustau A, Morris SJ, Makkerh J, Barker PA. K252a and CEP1347 are neuroprotective compounds that inhibit mixed-lineage kinase-3 and induce activation of Akt and ERK. *J Biol Chem* 2002;277:49473–80.