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Immunocytochemical evidence for the presence and location of the neurotrophin–Trk receptor family in adult human preovulatory ovarian follicles

David B. Seifer, MD,* Bo Feng, PhD, Robert M. Shelden, PhD

Department of Obstetrics, Gynecology and Reproductive Sciences, Division of Reproductive Endocrinology and Infertility, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ

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KEY WORDS
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Objective: This study was undertaken to evaluate the presence or absence of neurotrophins and their respective receptors within adult human preovulatory follicles.
Study design: Prospective study of neurotrophins and their receptors in follicular cells and unfertilized oocytes from women undergoing aspiration for in vitro fertilization/intracytoplasmic sperm injection. Cells (mural and cumulus granulosa cells, unfertilized oocytes) were examined for immunocytochemical staining of neurotrophin and receptor proteins.
Results: Mural and cumulus granulosa cells were positive for BDNF, NT-4/5, NT-3, and NGF, as well as for Trk B, Trk C, and Trk A receptors. Unfertilized oocytes were positive for Trk B, Trk C, and Trk A receptors.
Conclusion: Neurotrophins and their respective receptor proteins are present within the mural and cumulus granulosa cells of adult human preovulatory follicles. Neurotrophin receptors are present in human unfertilized oocytes. The location of the neurotrophins and their receptors suggest both an autocrine and paracrine function within the adult human ovarian follicle.

Development and maturation of vertebrate gametes is the culmination of a highly complex series of processes involving nonovarian (eg, hypothalamic releasing/stimulating factors, hypophyseal gonadotropin hormones), ovarian (steroid hormones), and cellular (cell signaling) inputs exquisitely orchestrated to produce gametes capable of fertilization. The oocyte carries the additional requirement for storing sufficient information and resources to support the newly formed zygote through 2 or more cell divisions until the newly emerging embryo genome is able to assume command of subsequent development.[1,2]

Although much remains to be elucidated, endocrine regulation of the hypothalamic-hypophyseal-gonadal axis through positive and negative feedback loops has been well established. Intragonadal and intracellular regulatory pathways and mechanisms are less well appreciated, but significant gains in understanding of

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* Reprint requests: David B. Seifer, MD, Maimonides Medical Center, 1355 84th St, Brooklyn, NY 11228.
E-mail: seiferdb@umdnj.edu

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these processes have occurred. It is, for example, well established through the work of Eppig and others[3-5] that the oocyte not only receives information and material from the surrounding granulosa cells (corona radiata), but also plays a highly significant reciprocal role in regulating the activity and production of these cells. Moreover, it is clear that a multitude of growth factors, eg, growth and development factor-9 (GDF-9), insulin-like growth factor (IGF), transforming growth factor (TGF-ß), bone morphogenetic protein (BMP)[6] and adhesion factors, eg, connexins,[7] are critical in the developmental processes involved in the intrafollicular oocyte-somatic cell communication network that leads to mature oocytes.

More recently, it has been recognized that neurotrophins, a family of soluble polypeptide growth factors well known for their role in neuronal survival and neural outgrowth, may be intimately involved in the dynamic development and maturation of the ovarian follicle/oocyte complex. Although other neurotrophic factors have been identified, the neurotrophins found in a variety of non-neuronal systems (eg, cardiovascular, immune, endocrine, and reproductive systems[8,9]) include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-4/5 (NT-4/5), and neurotrophin-3 (NT-3).

BDNF, NT-4/5, NT-3, and NGF and their respective tyrosine kinase (Trk) receptors (Trk B for BDNF and NT-4/5, Trk C for NT-3, and Trk A for NGF) have been identified in the mammalian ovary[10] and have been shown to play a role in ovulation,[11,12] steroid secretion,[13] and follicular development[14-16] in the rodent. Recently, we described presumptive evidence for the secretion of neurotrophins and the presence of their receptors in human cumulus cells.[17-20]

We first reported the presence of BDNF in the follicular fluid of the human ovarian follicle. We demonstrated its secretion by human cumulus granulosa cells and its promotion of mouse oocyte maturation presumably via its preferred receptor, Trk B.[17] We reported the presence of NT-4/5, also exhibiting preferential binding to the Trk B receptor, and NT-3, a non-Trk B ligand within the human ovarian follicle.[18] We further demonstrated the presence of Trk B receptor in the immature mouse oocyte and BDNF-induced acceleration of first polar body formation by cultured immature mouse oocytes.[17,18] It is emphasized that these prior studies examined the majority of neurotrophins secreted in specifically follicular fluid and cell culture media, not directly by cells per se, using commercially available enzyme-linked immunosorbent assays (ELISAs) with investigation of neurotrophin receptor presence limited to only the examination of mouse oocytes.[17,18]

In the current study, we extended these investigations to include direct immunocytochemical evidence of neurotrophins and their respective receptors in the basic cellular components of the human follicle that include mural granulosa cells, cumulus granulosa cells, and oocytes obtained from women undergoing oocyte aspiration for in vitro fertilization. Our objective was to determine whether neurotrophins and their respective receptors are present within the adult human preovulatory follicles of women undergoing follicular aspiration for in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI).

Material and methods

Population

Follicular fluid was obtained from 29 women younger than 42 years undergoing follicular aspiration for IVF/ICSI. Women had received either leuprolide acetate (Lupron, TAP Pharmaceuticals, Inc, North Chicago, IL) for pituitary desensitization, followed by 2 to 8 ampules of gonadotropins given daily in divided morning and evening doses as previously described[21] or daily gonadotropins until a lead follicle was 14 mm or greater in mean diameter whereupon cetrorelix acetate (Cetrotide, Serono, Norwell, MA) was added to prevent premature ovulation. Transvaginal follicular aspiration was performed under sedation 36 hours after administration of 5,000 or 10,000 IU hCG when at least 4 lead follicles were 18 mm or greater in mean diameter. The UMDNJ-Robert Wood Johnson Medical School Institutional Review Board approved this study protocol.

Human mural and cumulus granulosa cell culture

Pooled clear follicular fluid was centrifuged 10 minutes, $325\times g$ at 20°C. The top layer of cells enriched with mural granulosa was collected into Hanks' Balanced Salt Solution (HBSS, Ca and Mg-free, Sigma, St Louis, MO) then overlaid on top of 45% colloidal silica solution (Enhance-S Plus, Conception Technologies, San Diego, CA) and centrifuged for 20 minutes at $325\times g$. After separation, the mural granulosa cell enriched interface layer was collected and washed in HBSS. The resultant mural granulosa cell pellet was incubated in collagenase type IA (100 μ/mL, Sigma) at room temperature until cell clumps separated. Cells were washed twice then plated in 8-chamber slides (Nalge Nunc International Corp, Naperville, IL).

Cumulus granulosa cells were removed from oocytes in preparation for IVF/ICSI and then were incubated with hyaluronidase (100 IU/mL, Sigma), followed by 2× wash with HBSS. For each experiment, cumulus cells from 2 to 3 patients were pooled, then plated onto 8-chamber slides. Both cell types (mural and cumulus granulosa) were cultured in Ham's F-10 medium supplemented with 10% fetal bovine serum (FBS) at 37°C, 5% CO_2 in air. Each experiment was carried out a minimum of 3 times.