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TrkB agonist antibody ameliorates fertility deficits in aged and cyclophosphamide-induced premature ovarian failure model mice

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Premature ovarian failure (POF) is a leading cause of women’s infertility without effective treatment. Here we show that intravenous injection of Ab4B19, an agonistic antibody for the BDNF receptor TrkB, penetrates into ovarian follicles, activates TrkB signaling, and promotes ovary development. In both natural aging and cyclophosphamide-induced POF models, treatment with Ab4B19 completely reverses the reduction of pre-antral and antral follicles, and normalizes gonadal hormone. Ab4B19 also attenuates gonadotoxicity and inhibits apoptosis in cyclophosphamide-induced POF ovaries. Further, treatment with Ab4B19, but not BDNF, restores the number and quality of oocytes and enhances fertility. In human, BDNF levels are high in granulosa cells and TrkB levels increase in oocytes as they mature. Moreover, BDNF expression is down-regulated in follicles of aged women, and Ab4B19 activates TrkB signaling in human ovary tissue ex vivo. These results identify TrkB as a potential target for POF with differentiated mechanisms, and confirms superiority of TrkB activating antibody over BDNF as therapeutic agents.

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Premature ovarian failure (POF), also more commonly called primary or premature ovarian insufficiency (POI), is a leading infertility disease in humans, affecting 1–5% of women under age 40. Women with POF suffer from infertility, a series of menopausal symptoms, as well as some other severe complications such as psychological distress, osteoporosis, autoimmune disorders, ischemic heart disease, and even increased risk of mortality. Currently, there is no effective treatment for POF. Clinical care for women with POF involves hormone replacement therapy (HRT) plus psychological support or taking contraceptive drugs for those who are not desiring pregnancy. In addition to physical and emotional distress, the greatest unmet need for POF women of childbearing age is to bear children who are genetically their own.

The onset of POF has been attributed to premature follicle depletion due to accelerated atresia or altered maturation/primordial or primary ovarian insufficiency (POI). The pathogenesis of POF is poorly understood. The proposed mechanisms underlying POF vary from chromosomal or genetic alterations, infections, metabolic disorders to autoimmune diseases and iatrogenic factors (ovarian surgery, radiotherapy, chemotherapy, etc.). While there is no good genetic model that could be used for drug efficacy studies, two phenotypic models are often used: the “natural aging” model and the “chemotherapy-induced” model. Efforts for the treatment of POF have been directed towards holding ovarian reserve, activating dormant follicle, and alleviating follicle loss via potential protective agents (such as melatonin, etc.). Unfortunately, targets and mechanisms for these treatments are largely unknown, and few approaches described above could promote folliculogenesis of residual follicles in patients. Thus, these efforts are not very effective. One interesting study demonstrated that Akt-activating drugs could activate dormant primordial follicles and stimulate follicle growth. However, this treatment involves surgical removal of a piece of the ovary, fragmenting ovary into cubes for culture, followed by Akt stimulator treatment to activate primordial follicles. The activated donor ovarian cubes were then transplanted back to the recipient’s ovary, and subsequent retrieval of mature eggs for in vitro fertilization (IVF) is used. This study is very interesting scientifically, but the procedure is highly complicated and therefore its practical utility may be limited.

Brain-derived neurotrophic factor (BDNF), an extensively studied neurotrophic factor, is now known as an ovarian endocrine factor as well. Substantial evidence suggests that BDNF plays a role in ovarian follicle development. In mice, BDNF is expressed and secreted by granulosa cells (GCs) as well as cumulus cells (CCs). The receptor for BDNF, TrkB, is expressed in ovary tissues in two isoforms: a full-length TrkB (TrkB-FL) with an intracellular tyrosine kinase domain (ITK) for signaling, and a truncated one (TrkB-T1) that lacks ITK and is presumably non-functioning. Ovarian follicles at different stages are illustrated in Supplemental Fig. 7. TrkB-FL is primarily expressed in growing ovarian follicles. In contrast, TrkB-T1 is only expressed in primordial and primary follicles (granulosa cells and oocytes). Similarly, BDNF and TrkB have also been detected in human GCs/CCs, and oocytes respectively. The expression of TrkB-FL and TrkB-T1 in human ovary tissues has not been well documented.

Previous studies indicate that BDNF promotes oocyte maturation of mouse and other animals in vitro. Gene knockout experiments demonstrated that BDNF/neuropoetin-4-TrkB signaling is required for follicle growth and oocyte survival in vivo. The ovaries from both the conventional TrkB-null mice and newly developed mutant mice lacking all TrkB isoforms exhibited reduced numbers of follicles and abnormal ovaries, reduced GC proliferation, and decreased expression of FSH receptor (FSHR) in ovaries. Moreover, oocyte-specific deletion of the Ntrk2 (TrkB) gene revealed early adulthood infertility, with progressive post-pubertal depletion of oocytes accompanied by a loss of follicular organization. All these phenotypes are very similar to those seen in women with POF. In humans, the plasma level of BDNF is decreased in POF patients. Genome-wide association studies (GWAS) analysis have revealed a genetic association between Bdnf (11p14.1) and POF. Treatment with BDNF also promoted meiotic maturation in cultured immature human oocytes. Moreover, BDNF stimulated steroidogenesis and increased the proliferation of KGN cells (human granulosa-like cell line) by activating FSHR-mediated signaling.

Despite the association between dysregulation of BDNF-TrkB signaling and POF, preclinical and clinical studies suggest that BDNF itself cannot be used as a drug, because of its poor pharmacokinetics, limited diffusivity, and its activation of another receptor p75NTR, which often elicits effects different from or even opposite to TrkB. Thus, TrkB has never been considered as a drug target for the treatment of POF in previous studies. In all the patents published so far for TrkB agonists or antibodies, POF has never been listed as a disease indication.

Here, we show that a newly developed TrkB agonistic antibody (Ab4B19), with physicochemical properties superior to BDNF, can be used to treat POF. Ab4B19, delivered through tail vein injection, successfully engages its target TrkB in the ovary. In two different mouse POF models, Ab4B19 has the capacity to reverse the pathology of POF, rescue ovarian injury, and/or restore the number and quality of oocytes. Single-cell transcriptome analysis suggests that Ab4B19 may elicit similar effects in human cells. Our results support the notion that TrkB may be served as a drug target for POF and demonstrate that the TrkB agonistic antibody Ab4B19 could potentially be useful in treating POF, especially reverse infertility.

Results

Ab4B19 activated TrkB signaling in ovarian follicles. Blood-follicle-barrier (BFB), a molecular sieve with size- and charge-selectivity in ovarian follicles, is moderately permeable to mid-sized molecules, such as IgG1 (150 kDa), inter-alpha-trypsin inhibitor (Ixi1, 220 kDa), and fibrinogen (340 kDa). We sought to first examine whether the TrkB agonistic antibody Ab4B19 could penetrate into ovarian follicles by crossing BFB and activating TrkB signaling in the ovary. Anti-Müllerian hormone (AMH), a marker of granulosa/cumulus cells around the oocytes, was used to outline the ovarian follicles. Immunostaining was performed using a FITC-tagged anti-rabbit IgG secondary antibody to detect the rabbit monoclonal antibody Ab4B19 in follicles at different time points after its tail vein injection (1 mg/kg; iv) into adult mice. Ab4B19 was consistently present at the follicles at 24 h (Supplementary Fig. 1a), but not at 6 h, after its administration. The immune-reactivity was more abundantly located in granulosa cells (GCs) and oocytes at 48 h (Fig. 1a and Supplementary Fig. 1b). Moreover, we determined the penetration of Ab4B19 across the zona pellucida (Supplementary Fig. 1c). Immunocytochemistry using an anti-rabbit IgG antibody revealed that Ab4B19 not only bound to the surface of oocytes but also were endocytosed into the cytoplasm. Thus, Ab4B19 could penetrate into ovarian follicles in a time-dependent manner. Consistent with the above, the activation of TrkB downstream kinases, Akt1 and ERK1/2, as revealed by anti-pAkt and anti-pERK1/2 antibodies on Western blots, could be seen reliably in the initial 6 h after Ab4B19 administration (Supplementary
With the enrichment of Ab4B19 in ovarian follicles, TrkB signaling was markedly upregulated at 48 h (Supplementary Fig. 1d and Fig. 1b, c). In addition, the pharmacokinetic analysis indicated that the T1/2 for Ab4B19 (administered at 1 mg/kg) was approximately 3 days in the blood and ovary tissues (Fig. 1d). These results indicated the engagement of Ab4B19 with its target TrkB in the ovary, paving the way for its potential use for ovarian diseases.

**Ab4B19 promoted oocyte maturation and follicle development.** BDNF promoted oocyte meiosis in culture, as characterized by germinal vesicle (nuclear envelope) breakdown (GVBD), chromosome condensation, and extrusion of the first polar body. Our dose-response experiment using cultured oocytes revealed that similar to BDNF (0.2 nM), Ab4B19 (0.2 nM) effectively increased first polar body extrusion, but not GVBD (Fig. 2a, b), and this effect could be blocked by the Trk receptor inhibitor.
Fig. 1 Target engagement of Ab4B19 in ovarian follicles. a Immunofluorescence staining of ovarian follicles showed that Ab4B19 penetrated across BFB and co-localized with the AMH-positive granulosa cells in ovarian follicles. Ovaries were collected for immunostaining 48 h (48 h) after tail vein injection of Ab4B19 into adult mice. The rabbit monoclonal antibody Ab4B19 was detected with anti-rabbit IgG (FITC, green). AMH was probed with mouse anti-AMH and detected with anti-mouse IgG (TRITC, red). Cell nucleus were labeled with DAPI (blue). White arrows indicate the presence of Ab4B19 in ovarian follicles. Scale bar, 100 and 50 μm (the high magnification). Experiments were repeated three times independently with similar results (four sections/mice each time). b Time course of TrkB activation in the ovary, revealed by Western blots, after Ab4B19 administration (1 mg/kg; iv) into 8-week-old mice. Ovary tissues were collected and lysed at different time points after tail vein injection of Ab4B19, and pTrkB, pAkt, and pERK were analyzed. c Quantitative plots of TrkB activation and its downstream signaling (pAkt/Akt, pERK/ERK) at 48 h. N = 4 mice in the vehicle group and 5 mice in the Ab4B19 group, with the same samples repeated at least twice (n = 10). d Pharmacokinetics of Ab4B19 in plasma (left) and ovary tissues (right). Ab4B19 was administered into mice (2 months old, 1 mg/kg) by tail vein injection, its concentrations in ovary and plasma at different time points were analyzed by ELISA, and plotted against time (N = 3 mice per time point). Data were presented as mean ± SEM. Statistical analyses were carried out by two-tailed student’s t-test (*P < 0.05; **P < 0.01; ***P < 0.001). Vehicle: normal IgG injected. Source data are provided as a Source Data file.

K252a (Fig. 2c). We further determined whether Ab4B19 could promote oocyte ovulation under physiological conditions. After treating proestrus adult mice with Ab4B19 for 3 days, ovary tissues were sectioned and analyzed by H&E staining. Quantitative analysis indicated that there were a lot more corpora lutea in the Ab4B19-treated group, compared with the control (normal IgG treated, 1 mg/kg) group, suggesting that Ab4B19 promoted oocyte maturation (Fig. 2d).

Next, we examined the effect of Ab4B19 on ovarian follicle development using 15-day-old juvenile mice. The ovaries of these pre-pubertal mice contain primarily pre-antral follicles. Thus, the effect of Ab4B19 on pre-antral follicles could be easily determined by counting the follicle numbers. According to the accepted definitions, ovarian follicles were categorized as follows: primordial follicles, pre-antral follicles (primary and secondary follicles), and large growing (antral and preovulatory) follicles (See Supplementary Fig. 7 for follicles at various developmental stages). The ovaries were isolated and sectioned for H&E staining 5 days after a single dose of Ab4B19 (1 mg/kg). The representative images and quantification histogram of the different follicle populations are shown in Fig. 2e, f. Ab4B19 significantly facilitated the antral follicles development, but not primordial follicles and pre-antral follicles (Fig. 2f). Meanwhile, Ab4B19 also increased the level of AMH, which was produced by growing follicles ranging from primary to antral follicles (Fig. 2g). In addition, the expression of FSHR, which is required for follicle development and reflective of the biochemical differentiation of growing follicles, was also increased after Ab4B19 treatment (Fig. 2g).

We further determined the phosphorylation of FOXO3a, a key regulator of primordial follicle activation, as well as the expression of DDX4, an oocyte marker mainly expressed in primordial follicles, using 3-day-old ovaries cultured for 2 days. The western blotting analysis did not detect any change in pFOXO3a or DDX4 after Ab4B19 treatment (Supplementary Fig. 2a). Immunofluorescence revealed similar DDX4 staining in control and Ab4B19-treated ovaries (Supplementary Fig. 2b). These results suggest that TrkB activation by Ab4B19 contributed to the oocyte maturation and follicle development but had minimal effect on primordial follicles.

Ab4B19 attenuated ovarian degradation in NA-POF model. To determine whether Ab4B19 could be useful for POF therapy, we used the natural aging POF model (NA-POF), which mimics the conditions of progressive ovarian degradation. The schematic timeline of the Ab4B19 treatment is shown in Fig. 3a. We firstly measured whether the concentration of BDNF in the ovary changed as the mice got older. The level of BDNF protein, as determined by BDNF-ELISA, declined steadily from 2 months to 14 months, reaching 0.40 pg/mg protein in 10 months, about half of the level in 2-month-old mice (Fig. 3b). Pharmacodynamic (PD) analysis for the number of ovulated oocytes per mouse was performed using 12-month-old mice administered with Ab4B19 at various doses (0.1 mg/kg, 0.3 mg/kg, 1 mg/kg). In mice, the average estrous cycle is 4 days and the time from primordial follicle activation to ovulation is about 2–3 weeks. Given that PK (T1/2) of Ab4B19 (1 mg/kg) in blood and ovary was around 3 days (Fig. 1d), we decided to administer Ab4B19 through tail vein injection once every 4 days for 16 days. Administration of Ab4B19 to NA-POF (1 mg/kg) elicited a significant increase in ovulation, as measured by the number of ovulated oocytes per mouse (Fig. 3c). Similar to that in the young adult mice, the phosphorylation of Akt1 and Erk1/2 was also increased around 48 h after Ab4B19 administration to NA-POF (Supplementary Fig. 3a, b).

Next, we investigated the mechanisms by which Ab4B19 facilitates ovarian functions. Ab4B19 administration corrected the disrupted estrous cycle, which had been monitored for 12 days until they were caged with males (Fig. 3d), showing an "N" like cycling curve. Ab4B19-treated mice displayed more estrus stage (E), but not metestrus and diestrous stages (M/D) nor proestrus stage (P), as revealed by microscopic analysis of the predominant cell types in the vaginal smears (Fig. 3e). Ab4B19 also improved the morphology and increased the follicle number of the ovary in the NA-POF mouse model (Fig. 3f). Quantitative analyses indicated that Ab4B19 also reduced the atretic follicles (Fig. 3h), increased the number of corpora lutea, and restored the development of pre-antral follicles and antral follicles, without affecting primordial follicles (Fig. 3g). Treatment with Ab4B19 also reversed abnormality in blood E2 hormone (Fig. 3i). Interestingly, it did not affect the levels of FSH hormone (Fig. 3j). Finally, the expression of AMH and FSHR was also upregulated after Ab4B19 treatment (Fig. 3k). Taken together, Ab4B19 could halt multiple aspects of ovarian degradation in NA-POF mice.
including body weight and appearance was done for these newborn mouse pups. The fertility index, namely the ratio of females delivered offspring/total females, was up to 38.7% for Ab4B19-treated group, and 14.2% for the vehicle-treated group. The average number of newborn pups per mouse reached 3.6 (ranged from 0 to 6) for the Ab4B19-treated group, but only 1.1 (ranged from 0 to 3) in the vehicle-treated group (Fig. 4e). The newborn pups (age 2-day) derived from Ab4B19-treated mothers had normal body weight (Fig. 4f), and exhibited no malformation, compared with control pups derived from vehicle-treated mothers. Interestingly, the analyses of embryo implantation and litter size indicate that the number of actual birth was generally lower than the number of embryos implanted in the uterus, due most likely to intrauterine abortion or absorption of embryos.
These results together demonstrated the beneficial effects of Ab4B19 on the fertility in the NA-POF model.

Next, a series of safety evaluation experiments were performed in not only the first generation (those derived from Ab4B19-treated mothers) but also the second generation (those derived from males or females whose mothers were treated with Ab4B19) of offspring mice. Supplementary Table 1 summarizes the fertility parameters, which include fertility index, estrous cycle, number of pups born, and mean body weight, for the first-generation mice at 7–8 weeks of age. These pups were completely normal in all these parameters. Supplementary Table 2 shows blood test results, which include cholesterol, glucose, FSH, LH, as well as the morphology of testis, ovary, liver, etc., of the first-generation mice, and the mice from control mothers, at the same age. No difference was found between the two groups. Supplementary Table 3 and Supplementary Table 4 summarize the fertility parameters and blood test results for the second-generation mice at 7–8 weeks of age. Again, the offspring derived from Ab4B19-treated grandmothers were almost the same as those derived from control grandmothers. Finally, we assessed whether the Ab4B19 treatment affected the cognitive functions of the offspring. In the novel object recognition test, there was no difference between the first generation of offspring and those derived from control mice, in either exploration time or discrimination ratio, during both acquisition and test periods (Fig. 4g). Safety evaluation of Ab4B19 in either exploration time or discrimination ratio, during both treatment affected the cognitive functions of the offspring. In the treated grandmothers were almost the same as those derived from BDNF-TrkB pathway.

Ab4B19 alleviated ovarian injuries in the Cy-POF model. Premature ovarian failure followed by infertility is one of the most severe side effects of chemotherapies in young female cancer patients, but there is no effective treatment for the gonadotoxic damages in their ovary. Cyclophosphamide (Cy) is a commonly used chemotherapeutic that could induce severe ovary damage and is a recognized risk for POF. Previous reports showed that the depletion of primordial follicles induced by Cy treatment could last for more than 20 days. We also found that the number of primordial follicles in Cy-treated animals at 13 days was significantly reduced than that at 7 days after Cy induction in mice (Supplementary Fig. 4a). Therefore, a Cy-induced mouse model (Cy-POF) was used to assess the therapeutic efficacy of Ab4B19 on preserving primordial follicles 7 days after Cy induction in this study. Figure 5a schematizes the protocol and timeline for generating Cy-POF mice and Ab4B19 treatment. A single dose of Cy (75 mg/kg, 200–300 μl) was administered intraperitoneally to young female mice (6–8-week-old, C57BL/6). After a 7-day induction, the mice were treated with Ab4B19 or vehicle for 6 days (1 mg/kg, iv, once every 3 days). Note that the dosing interval was shortened to 3 days because more drug exposure will benefit cell survival in this acute injury model. H&E staining (midline sections) of ovaries revealed that Ab4B19 treatment significantly alleviated the Cy-induced damage to ovarian follicles, resulting in their normal development (Fig. 5b). Quantitative analysis indicated that Ab4B19 treatment attenuated the Cy-induced reduction in the number of primordial follicles, as well as those of the early growing follicles, antral follicles (Fig. 5c). Ab4B19 also reversed the Cy-induced increase in atretic follicles (Fig. 5c). Cy-treated ovaries also exhibited a reduced ratio of corpora lutea, representing less successful ovulation, and this was also rescued by Ab4B19 treatment (Fig. 5c).

While mechanisms underlying chemotherapy-induced ovarian damage are not fully understood, its direct toxicity to growing follicles by apoptosis is apparent. Western blotting revealed that Ab4B19 treatment increased the ratio of BCL2 to BAX protein level (Supplementary Fig. 4b) and reduced the expression of cleaved-caspase-3 (Fig. 5d), suggesting inhibition of Cy-induced apoptosis. Immunostaining of cleaved-caspase-3 identifies apoptotic follicles. The fluorescence signals were dramatically increased in the ovarian sections derived from Cy-POF mice, but not in those from POF mice treated with Ab4B19 (Fig. 5e). Quantification of follicles in different developmental stages indicated that the apoptotic index (caspase-3-positive follicles/total follicles) in pre-antral and early antral stages were rescued in Ab4B19-treated ovaries, compared with the vehicle-treated ovaries (Fig. 5e, f).

Ab4B19 reversed infertility in the Cy-POF model. We next examined the effect of Ab4B19 on fertility in Cy-POF mice. Because of Cy-induced gonadotoxic damage to the ovary, the expression of AMH was decreased in Cy-treated ovary compared with control ovary, but such a decrease was reversed after Ab4B19 treatment (Fig. 6a, b and Supplementary Fig. 4c). Cy-induced injury in ovaries was accompanied by an increase in FSH but a decrease in E2 hormone in the serum. Remarkably, Ab4B19 treatment reversed the levels of these two hormones close to normal levels (Fig. 6c, d). Moreover, the Cy-POF mice exhibited a reduced number of ovulated oocytes and an increased proportion of abnormal oocytes, and treatment with Ab4B19 reversed these changes as well (Fig. 6e–g). By comparison, with the same dose but more dosing times than Ab4B19, treatment with BDNF failed to elicit an increase in the number of ovulated oocytes or decrease...
in the proportion of abnormal oocytes (Supplementary Fig. 5), demonstrating the superiority of Ab4B19 over BDNF as a therapeutic agent for Cy-POF.

To further determine whether Ab4B19 improved the fertility of Cy-POF mice, mating trials were performed. It has been shown that treatment with 75 mg/kg Cy led to a short-term decrease in fertility because of a decreased number of growing follicles, but the fertility returns to normal due to a continuous supply of the remaining primordial follicles. In the longer term, the fertility of mice would decline again due to the gradual exhaustion of primordial follicles. It is therefore important to choose the right time window to reveal the fertility changes in the Cy-POF model. We mated Ab4B19-treated female mice with untreated fertile males 3 days after the final dosing of Ab4B19 (1 mg/kg, once every 3 days for 6 days, Fig. 5a), a time window in which Cy should elicit its clear decrease in fertility (i.e., 7 days after treatment with Cy). We found that all female mice were successfully mated within
1 week after the male mice were introduced and got pregnant (Supplementary Table 6). However, the Cy-POF mice exhibited a reduced litter size (numbers of pups per litter), compared with the control mice. Importantly, this number was reversed by Ab4B19 treatment (Fig. 6h). To further examine the long-term protective effect of Ab4B19 on the primordial follicle pool, these female mice were mated a second round ~5 weeks after the first-round parturition. Treatment with Ab4B19 again resulted in a significant increase in fertility in the Cy-treated female mice when they were mated in the second round (Fig. 6i). On average, there were ~10 babies/mother for Cy, and 9 babies/mother for Cy-Ab4B19 groups in both first and second rounds, indicating clearly that the TrkB antibody treatment improved fertility. Moreover, the six female mice in the Ctrl group had given altogether 21 times of birth, averaging 3.5 births/mouse before termination (in 9 months). In Cy-POF females, the Vehicle and Ab4B19-treated groups actually had completed 15 times of birth (15/6 = 25 births/mouse) and 18 times of birth (18/6 = 3 births/mouse) before termination, respectively. Thus, Ab4B19 not only increased the number of pups/birth but also the number of births in their adult lives (9 months). These results suggest that the gonadotoxic damage of growing follicles in the Cy-POF model, and both the short-term and long-term defects of fertility could be well rescued by Ab4B19 treatment.

**Effects of Ab4B19 on human ovarian cells.** BDNF has been shown to modulate granulosa cell proliferation and steroidogenesis in mice by activating the FSHR/cAMP/PKA/CREB signaling. It is imperative that the utility of Ab4B19 be examined in human ovarian cells. Due to the difficulties in obtaining ovarian tissues from aged women or POF patients, the human granulosa cell line (KGN cells) was employed. KGN cells, established by long-term culture of human ovarian granulosa cell carcinoma, have steroidogenic activities and express functional FSH receptors. Thus, this cell line is considered a very useful model to explore the physiological regulation of human granulosa cells. We found that Ab4B19 at a concentration as low as 0.2 nM promoted the survival of KGN cells, as measured by Cell Counting Kit-8 (CCK-8) (Fig. 7a). Application of Ab4B19 to KGN cells in culture also stimulated CREB phosphorylation after 24-h treatment (Fig. 7b). These results provide preliminary evidence for the efficacy of Ab4B19 on human ovarian cells.

To further explore the clinical relevance of TrkB activation in POF, we reanalyzed our single-cell RNA-seq experiments for BDNF and TrkB mRNA expression in human ovarian follicles. Data from human oocytes and corresponding granulosa cells (GCs) spanning five follicular stages were obtained. The dynamic expressions of BDNF and TrkB transcripts were examined using principal component analysis (PCA) (Supplementary Table 7). We found that BDNF transcripts were expressed in GCs of all five follicular stages, with an increased expression level in oocytes from secondary to antral follicles (Fig. 7c). More importantly, the expression pattern of the two TrkB isoforms in human oocytes and GCs suggests a similar functional mechanism in the ovary. TrkB-T1 mRNA expression exhibited a gradual decline during development in GCs (Fig. 7d, left) and oocytes (Fig. 7e, left). In contrast, the expression of TrkB-FL mRNA experienced a transient upregulation in GCs of primary follicles (Fig. 7d, right) but a sustained increase in oocytes from the stage of secondary follicles (Fig. 7e, right). Thus, the changes in the expression of TrkB-T1 and TrkB-FL in human oocytes are similar to those in mice.

To determine the expression of BDNF protein and the impact of aging, we performed BDNF immunostaining with ovarian sections derived from young and aged females. In clinics, POF patients seldom undergo surgery. Here the young (ages 29–35) and aged (61–64) female groups were used as the high- and low-fertility group respectively. It is generally believed that the follicle number is dramatically reduced after menopause. However, there are occasional reports that at age 60 or even 70, women could still get pregnant, suggesting that some of their follicles might still be functional after amenorrhea. With careful examination, we could identify a few small follicles (with diameter less than 100 μm) with clear structural characteristics of primordial or primary follicles and co-labeling with an established marker DDX4/MVH (Fig. 7f). We found that BDNF expression in follicles from aged women is significantly lower than that in follicles from young ones (Fig. 7f, g and Supplementary Fig. 6b). The levels of BDNF protein from individual samples are shown in Supplementary Fig. 6a. To further investigate whether Ab4B19 could increase TrkB activation in human tissues, we performed an ex vivo experiment. Human ovarian tissues with visible follicles were dissected from female donors who underwent surgery for endometrial stromal sarcoma, fragmented into small cubes, and incubated in 48-well tissue culture plates with culture medium at 37 °C. Then they were treated with 1 nM BDNF, 3 nM normal IgG, or 3 nM Ab4B19 for 35 min and lysed for Western blotting analysis. Similar to BDNF, treatment with Ab4B19 elicited a marked increase in phosphorylated Erk, a sensitive downstream signal of TrkB activation (Supplementary Fig. 6c, d). These results reveal the translational potential of the present study: Ab4B19 may have similar therapeutic effects in humans as those in the mouse models.
Discussion
As of today, there is no FDA-proved drug for POF, despite huge unmet needs. In this study, we have provided evidence that an agonistic antibody (Ab4B19) targeting the BDNF receptor TrkB in ovarian follicles could be a promising strategy for POF treatment. Ab4B19, which is superior to BDNF in diffusibility, PK and receptor specificity, activated TrkB and its downstream signaling in ovary, and promoted oocyte maturation and ovarian follicular development. In the natural aging-induced mouse model (NA-POF), treatment with Ab4B19 rescued ovarian degradation and infertility. In the cyclophosphamide (Cy) induced POF model (Cy-POF), Ab4B19 repaired ovarian damage,
Fig. 5 Alleviation of ovarian defect and gonadotoxicity by Ab4B19 in Cy-POF model. a Experimental design of Ab4B19 treatment and analysis in Cy-POF mice. b H&E staining ovaries (midline sections) harvested from a normal female mouse (left) or a Cy-POF mouse treated with vehicle (center) or Ab4B19 (right) for 6 days. Asterisks: the corpora lutea; Red arrow: atretic ovarian follicle. Scale bars, 200 μm. c Quantification of primordial, pre-antral, antral follicles, atretic follicles, and corpora lutea per ovary in normal mice (Ctrl), and Cy-POF mice treated with vehicle or Ab4B19. N = 8 ovaries per each condition. Unless stated otherwise, statistical analyses for comparison among three or more groups in this and all the following figures were carried out using one-way ANOVA, followed by Dunnett’s multiple comparisons tests. d Western blotting showing the expression of cleaved and total caspase-3. The apoptotic level was determined by the normalized ratio of the cleaved to total caspase-3. N = 5 mice per group. e Immunofluorescence images of ovaries stained with cleaved caspase-3 antibody (Green: cleaved caspase-3, Blue: DAPI). Images in the red frames in the center row are magnified and shown on the right. Scale bars, 200 μm. f Quantification of apoptosis in pre-antral and antral follicles. Apoptosis index was expressed as apoptotic/total follicles (N = 5 mice per group). Data were all presented as mean ± SEM. Statistical analyses were carried out using one-way ANOVA followed by the Dunnett’s multiple comparisons test (c, d, f) (*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001). Source data are provided as a Source Data file.
inhibited follicular apoptosis, and restored the number and quality of oocytes. The potential effects of Ab4B19 on ovarian follicles at various developmental stages, as well as those on the NA-POF and Cy-POF models, are summarized in Supplemental Fig. 7. Further, single-cell RNA-seq analysis of human ovarian cells revealed a significant presence of BDNF in GCs and an increased expression of TrkB in oocytes as they mature. Ab4B19 could also induce TrkB signaling and enhance survival in cells derived from human GCs. These results together have demonstrated the beneficial effects of Ab4B19 on animal POF models and provided a foundation for further testing its therapeutic potential in treating human POF.

Given the lack of therapy for POF, current treatment strategies have focused on indirect or symptomatic treatments, such as alleviating follicle loss or restoring hormonal balance\(^{10,16}\). These approaches are generally not very effective. Hormone replacement therapy (HRT) could reduce some of the POF-associated complications and improve the quality of life for women with POF\(^{52}\). Unfortunately, these treatments are accompanied by an increased risk of cancer\(^{34,55}\). Moreover, there is no standard HRT regimen, which should be individualized according to the diagnosis\(^{4,5}\). Some protective agents, such as melatonin, sphingosine-1-phosphate (S1P), ceramide-1-phosphate (C1P), etc., appear to exert positive effects on gonadotropin recovery and restore fertility. How these agents achieve their "protective" effects remain a mystery. One limitation of S1P, an inhibitor of ceramide-promoted cell death, is that it must be injected directly into the ovary to avoid disturbing other physiologic mechanisms\(^{56}\). Stem cell therapy has been controversial because of safety concerns on stem cell transplantation into women's ovary\(^{10}\). Most importantly, none of the treatments highlighted above have a clear mechanism or target for the illness, making it difficult for further development into effective medicines.

The TrkB antibody strategy used in the present study may have several unique advantages, in comparison with the existing treatments. First, expression studies, cell culture work, and in vivo animal work have all supported the rationale for the protection/
repair mechanism mediated by the BDNF-TrkB signaling pathway. Second, Ab4B19 reverses infertility by preserving folliculogenesis in two animal models of POF. Third, Ab4B19 also exhibits excellent drug-like properties, overcoming the shortcoming of BDNF. Finally, Ab4B19 did not induce any visible side effects in mice. Therefore, Ab4B19 has demonstrated its potential to be a candidate drug.

Once diagnosed, POF patients usually display infertility, a devastating consequence of the disease. These patients have a very low probability of spontaneous pregnancy and do not respond to traditional therapies. However, most of the POF cases are not true "ovary failures": nearly 75% of women with POF have detectable follicles in their ovary. The animal models in our study could not represent all types of human POF/POI, which is a heterogeneous...
disorder with 70% idiopathic. Moreover, POF patients who had been through chemotherapy (10–15%) for several years may also be different from Cy-POF mice with acute onset. It would be interesting to determine the effect of Ab4B19 on mice that had been treated with Cy for different lengths of time. The main goal of the present study was to demonstrate the feasibility of our therapeutic strategy: to promote the residual follicular development and maturation with the TrkB agonistic antibody and protect damaged follicles (such as Cy-induced POF).

Our results imply that TrkB antibody can be used to treat different types of POF/POI patients regardless of their etiology or onset time, as long as they have residual follicles. Whether this strategy is truly effective in certain subtypes of POF patients will need to be tested through clinical trials. The NA-POF mice exhibit the ovarian recession with a decrease, but not elimination, in the number of ovarian follicles and a low-fertility rate, leading to a continuous loss of primordial follicles. The number of primordial follicles in the Cy-treated ovaries would always be higher than that in untreated ovaries, resulting in a “burnout” effect and ovarian follicle depletion. Given that primordial follicles express primarily the non-functional TrkB-T1 but not TrkB-FL, it is less likely that Ab4B19 attenuates the P13K-Akt pathway in the primordial follicles per se. Extensive studies suggest that the AMH level is critical for maintaining the inactive state of primordial follicles. Previous studies have also shown that AMH prevents the over-activation of primordial follicles in Cy-POF mice. In this study, we found that Cy treatment induced a significant reduction in various developing follicles and AMH levels, leading to a continuous loss of primordial follicles. The number of primordial follicles in Cy-treated animals at 13 days was significantly reduced than that at 7 days after Cy induction in mice (Supplementary Fig. 4a). Thus, the consumption of primordial follicles in Cy-treated ovaries would always be higher than that in untreated ovaries, until the AMH level in Cy-POF mice returns to normal, and the duration may be much more than 13 days. Our results that the delayed administration of Ab4B19 (7 days after Cy treatment) partially rescued the ovarian dysfunction in Cy-POF mice indirectly verify that there is secondary Cy-induced damage. Similar experiments in previous reports also showed a long-lasting depletion of primordial follicles induced by Cy treatment: (1) Depletion of primordial follicles induced by 50 mg/kg Cy lasted for at least 20 days in 18-week-old mice; (2) Depletion of primordial follicles caused by 150 mg/kg Cy treatment on day-21 was much more obvious than that on day-7 (~45 vs. 20%). We, therefore, propose that Ab4B19 may regulate primordial follicles (Fig. 5c) indirectly by maintaining AMH levels, which were elevated in growing follicles in the entire ovary (Fig. 6a, b). In addition to P13K-Akt mediated “burnout” (direct) and disinhibition by AMH reduction (indirect), several other pathways that induced indirect effects on primordial follicle pools were also summarized by Spears et al. Further work is necessary to delineate how Ab4B19 regulates primordial follicles.

As a candidate drug for pre-pregnancy women, TrkB agonistic antibody must be carefully evaluated for its safety. During the entire course of Ab4B19 treatment (1 mg/kg, 16 days, once every 4 days), we could not observe any malformation in the animals, except lower body weight after Ab4B19 treatment, a phenomenon
consistently observed in previous studies using TrkB agonistic antibodies. For safety evaluation on embryos, we found no abnormalities in 8-day-old embryos when we examined the implantation sites in the uterus. Moreover, the concentration of Ab4B19 during the period of embryo implantation (about 9 days after its final dosing) was reduced to a very low level (down to about 10%), which may not be sufficient to activate TrkB signaling. Further, health assessments, as well as a detailed examination of the reproductive system of the mice derived from Ab4B19-treated mothers, showed no anomalies. We examined the fertility, as well as some biochemical indices and the gonadal hormones in the blood, in the first- and second-generation offspring mice. There is no visible body malformation and weight change in these mice. Given the important roles of BDNF-TrkB signaling in the brain, we examined the effect of Ab4B19 on novel object recognition, a cognitive function known to be regulated by BDNF, in the first-generation offspring mice. Again no anomaly was found. A formal GLP toxicology study will need to be performed for a complete safety assessment before Ab4B19 proceeds for clinical studies.

An important question that remains to be answered is whether the preclinical discoveries on Ab4B19 are translatable in human POF. Genetic association studies have identified a link between Bdnfm and POF. A reduced level of BDNF protein has been reported in the plasma of menopausal women and POF patients. However, previous human studies were fragmented and circumstantial, and there was no evidence that the phenotypes of TrkB knockout mice are translatable. Our single-cell RNA-seq showed relatively high levels of BDNF expression in follicles of all stages of the human ovary, indicating its role throughout follicle development. However, BDNF expression is downregulated in the ovaries of aged women (Fig. 7f, g and Supplementary Fig. 6a, b). Further, the expression pattern for TrkB-FL mRNA, which is similar to that in mice, suggests that BDNF-TrkB may function transiently in primary/secondary follicle stages for GCs but continuously from the secondary follicle stage on for oocytes in humans. Finally, Ab4B19 elicited better effects than BDNF on CREB phosphorylation and cell survival in human-derived KGN cells (Fig. 7a, b). More importantly, Ab4B19 could activate TrkB downstream signaling in human oocyte tissue ex vivo (Supplementary Fig. 6c, d). These human data reveal the translational potential of Ab4B19 for POF.

In summary, Ab4B19 not only extends and enhances the reproductive capability in the natural aging mouse but also protects the fertility and repairs the developing follicles’ damage in the Cy-treated mice. These results identified a potential first-in-class drug for clinical therapy of POF, with differentiated target/mechanism and novel chemical entity.

Cell lines culture and proliferation assay. KGN cells kindly donated by Dr. Yi-Ming Mu (Chinese PLA General Hospital, Beijing, China) were cultured as previously described. Eagle’s Minimum Medium (DMEM)/1% FBS medium, supplemented with 10% fetal bovine serum (Gibco, USA), 1% penicillin/streptomycin (Gibco, USA). To determine the effects of BDNF and Ab4B19 on KGN cells, Cell Counting Kit-8 (CCK-8) (402003576, YEASEN) assay was used to assess cell proliferation. In brief, KGN cells were seeded on 96-well plates. After 24 h incubation in culture medium, BDNF (0.2 nm) or Ab4B19 (0.2, 0.6, or 1.8 nm) was added into wells. After 72 h treatment, 10 μl CCK-8 solution was added into each well and incubated at 37 °C for 2 h. The absorbance at 450 nm was determined using a microplate reader (Biotek, Cytation5). The cell viability was calculated by the optical density (OD) values of treated groups/OD values of the control group × 100%.

Ovary culture. Ovaries were dissected from 3-day-old female pups and cultured in 24-well tissue culture plates coated with agarose gel at 37 °C in an atmosphere of a 5% CO2/95% air incubator. For the natural aging POF (NA-POF) model, female (15 days, 6-8 weeks old) C57BL/6 mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. and housed in the specific pathogen-free (SPF) condition with temperature control (22 ± 1 °C) and humidity control (60 ± 10%) on a 12 h light/12 h dark cycle with ad libitum access to water and regular rodent chow. All the animal protocols were approved by Tsinghua University Animal Care and Use Committee (Protocol number: 17-LB17). For the experiments with 15-day-old mice, they were randomly treated with Ab4B19 and Normal IgG through tail vein injection for 5 days respectively. For the natural aging POF (NA-POF) model, female C57BL/6 mice of 6-8 months old were purchased. After acclimation until 12 months old, the mice were divided into two groups randomly and treated with Ab4B19 or Normal IgG (1 mg/kg, once every 4 days) through tail vein injection for 16 days.

For cyclophosphamide (Cy)-induced POF (Cy-POF) model, 6-8 weeks old C57BL/6 female mice were weighed and then treated with a single intraperitoneal injection of Cy (75 mg/kg, 200–300 μl), or an equal volume of saline solution as the control. Seven days later, these mice were divided into three groups (n = 12 each) randomly for three different treatments: control (Ctrl), POF + Normal IgG (Vehicle), POF + Ab4B19 (Ab4B19).

Methods

Animal model. Female (15 days, 6-8 months, and 6-8 weeks old) C57BL/6 mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. and housed in the specific pathogen-free (SPF) condition with temperature control (22 ± 1 °C) and humidity control (60 ± 10%) on a 12 h light/12 h dark cycle with ad libitum access to water and regular rodent chow. All the animal protocols were approved by Tsinghua University Animal Care and Use Committee (Protocol number: 17-LB17). For the experiments with 15-day-old mice, they were randomly treated with Ab4B19 and Normal IgG through tail vein injection for 5 days respectively.

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Serum analysis. After Ab4B19 administration, the blood samples were collected from the mice orbital sinus under anesthesia with avertin. After clotting at room temperature for 90 min, serum was obtained via centrifuging at 1800 × g and stored at −80 °C for further analysis. The serum levels of E2 and FSH were analyzed using the standard protocols of the ELISA kits (Cloud-clone, Wu Han). The levels of testosterone, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were determined with 125I-labeled RIA kits (Beijing North Institute of Biological Technology). BDNF levels in analyzed following the instruction of different kits measured using the standard protocol of the ELISA kit (Genstar, C643-01).

Vaginal smears and estrous cycle determination. After the second treatment with Ab4B19, estrous cyclicity was monitored by vaginal smears taken daily at the same time each day over at least three consecutive estrous cycles (12 days). In brief, saline was infused into the vagina using the pipette and collected in a 1.5 ml tube, followed by transferring to a glass slide for microscopic analysis. The estrous cycles were determined by the vaginal cytology of dominant cell type: proestrus stage: round, nucleated epithelial cells; estrus stage: cornified squamous epithelial cells; metestrus stage: epithelial cells and leukocytes; and diestrus stage: nucleated epithelial cells and a predominance of leukocytes.

Western blotting. KG cells, mouse, or human ovarian tissues were lysed with the lysis buffer (20 mM HEPES,150 mM sodium chloride, 1.0% CA-630, 0.1% SDS, 2 mM Ethylenediaminetetraacetic acid, 1% deoxycholic acid, protease, and phosphatase inhibitor mixture (Roche Diagnostics)). Protein concentrations were measured by BCA kit (Thermo Scientific). Thereafter, proteins were denatured at 98 °C for 3 min followed by SDSPAGE PAGE (10% or 15% SDS-PAGE gel). After transferring proteins onto PVDF membranes (BIO-RAD) followed by incubation with blocking buffer for 1 h at room temperature, the membranes were incubated with the primary antibodies in blocking solutions at 4 °C overnight before detection with HRP-conjugated secondary antibodies (Cell Signaling Technology, #7074, 1:10000). After the membranes were washed, proteins were revealed by enhanced chemiluminescence Substrate (Thermo Scientific) by Tanon 5200 (software: Tanon MP, v1.02) and finally displayed on the gel imager for grayscale analysis using Tanon Gis (v4.2). The following primary antibodies were used: anti-TrKB (Rabbit, CST, 1:1000), anti-Phos-TrKB (Rabbit, CST, 1:1000), anti-Akt (Rabbit, Easybio, 1:1000), anti-p-Akt (Rabbit, CST, 1:2000), anti-CREB (Rabbit, CST, 1:1000), anti-p-CREB (Rabbit, CST, 1:2000), anti-ERK (Rabbit, CST, 1:1000), anti-p-ERK (Rabbit, CST, 1:2000), anti-DDX4 (ab13840, Abcam, 1:1000), anti-phosphoOxO3a (9464, CST, 1:1000) anti-BAX (ab32503, Abcam, 1:200), anti-Bcl-2 (sc-7382, Santa Cruz, 1:800), anti-cleaved CASP3 (9664, CST, 1:1000), anti-AMH (Rabbit, Abcam, 1:1000) anti-FSHR (Rabbit, Biocell, 1:1000), anti-GAPDH (Mouse, Easybio, 1:1000), anti-β-actin (AA128, Beyotime, 1:1000).

Superovery and oocyte morphology analysis. For the fertility evaluation, oocyte superovulation is carried out for all experimental groups, which were treated by an injection (i.p.) of pregnant mare serum gonadotrophin (PMSG, 5IU), followed by the administration (i.p.) of hCG (5UI) 48 h later. Oocyte-cumulus complexes were collected from the ampulla 13–14 h after hCG administration. Oocytes were counted after enzymatic dissociation from the surrounding cumulus with a solution of 10 mg/ml hyaluronidase (Millipore-Sigma). The evaluation of oocyte quality was performed according to the morphological criteria from a previous review. Mouse oocytes are classified into: (1) normal oocytes, (2) abnormal oocytes (cytoplasmic abnormalities (thick or hollow oocyte and large perivitelline space), or intracytoplasmic abnormalities (dark or granular cytoplasm and cytoplasmic fragments), or shape abnormalities.

Fertility assessment. For the fertility assessment, breeding examination and detection of embryos’ implantation sites on pregnant mouse uteri were carried out. Female mice were housed with identifiably fertile C57BL/6 males at a 1:1 ratio. Successful mating was judged by observation of a vaginal plug. For NA-POF mice, successful mating was judged by observation of a vaginal plug and stored out. In total, 80 oocytes and 71 GCs at 35 min and harvested for Western blotting analysis. Since the ovarian pieces were dissected from female donors who underwent surgery for endometrial stromal sarcoma, removed of fibrous tissues of the tunica albuginea and some fatty tissues, fragmented into small cubes, and incubated in 48-well tissue culture plates with culture medium at 37 °C in a 5% CO2 incubator. Then they were treated with 1 nM BDNF, 3 nM normal IgG, or 3 nM Ab4B19 for 35 min and harvested for Western blotting analysis. Since the ovarian pieces were mixed and divided into three groups, a similar number of follicles were distributed in each group. The culture medium was DMEM/F12 (Gibco, USA) and a Minimum Essential Medium (MEM, Hyclone, SH30265.01) (1:1) supplemented with 10% fetal bovine serum (Gibco, USA), 1% penicillin/streptomycin (Gibco, USA).

Novel object recognition. The novel object recognition test (NORT) was conducted under dim light (~6.5 lux) in a plastic open-field box (40 cm length × 40 cm width × 30 cm height). A digital camera was installed above the box to record the behavior of the animals. Mice were habituated to the testing room and the behavioral box at 10 a.m. for 3 consecutive days before testing (30 min for the testing room and 5 min for free exploration of the empty box). During the acquisition phase, a mouse was allowed to freely explore the two identical objects (bottles) for 5 min. During the test phase, one of the two objects was replaced by a novel object (woody brick) and the mouse was allowed to freely explore for 10 min. The amount of time that the mouse spent exploring the novel and familiar objects was counted, respectively. Exploration time and the discrimination ratio of the acquisition phase and test phase were presented. The discrimination ratio was calculated as score = (time spent with novel object – time spent with identical object)/(total time spent with both objects).

Statistics. Statistical analyses were performed with the software GraphPad Prism (GraphPad, v7.0). Statistical tests and P values are reported in the text and figure legends. Data were presented as mean ± SEM. Significance was defined as *P < 0.05, **P < 0.01 and ***P < 0.001.

Data availability
All relevant data and code are available within the article and its supplementary information/Source data or freely available from the corresponding authors upon reasonable request. RNA-Seq data is from the NCBI Gene Expression Omnibus (GEO) under the accession number: GSE107746. RNA-Seq data for BDNF and TrkB expression (Fig. 7e–c) are also available in Source data. Source data are provided with this paper.

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References
1. Goswami, D. & Conway, G. S. Premature ovarian failure. Hum. Reprod. Update 11, 391–410 (2005).
2. De Vos, M., Devroey, P. & Fauser, B. C. Primary ovarian insufficiency. Lancet 376, 911–921 (2010).
3. Welt, C. K. Primary ovarian insufficiency: a more accurate term for premature ovarian failure. Clin. Endocrinol. 68, 499–509 (2008).
4. Pol, E. G. Go. et al. ESHRE Guideline: management of women with premature ovarian insufficiency. Hum. Reprod. 31, 926–937 (2016).
5. Nguyen, H. H., Milat, F. & Vincent, A. Premature ovarian insufficiency in general practice: meeting the needs of women. Aust. Fam. Physician 46, 360–366 (2017).
6. Persani, L., Rossetti, R. & Cacciatore, C. Genes involved in human premature ovarian failure. J. Mol. Endocrinol. 45, 257 (2010).
7. Jiao, X., Ke, H., Qin, Y. & Chen, Z.-J. Molecular genetics of premature ovarian insufficiency. Trends Endocrinol. Metab. 29, 795–807 (2018).
8. Kalich-Philosoph, L. et al. Cyclophosphamide triggers follicle activation and “burnout”; AS101 prevents follicle loss and preserves fertility. Sci. Transl. Med. 5, 183ra162–183ra162 (2013).
9. Song, G., Beaufay-Hendryberg, M. & Binart, N. AMH prevents primordial ovarian follicle loss and fertility alteration in cyclophosphamide-treated mice. FASEB J. 33, 1278–1287 (2019).
10. Sheikhansari, G., Aghabati-Maleki, L., Nouri, M., Jaddi-Niafragh, F. & Yousefi, M. Current approaches for the treatment of premature ovarian failure with stem cell therapy. Biomed. Pharmacother. 102, 234–262 (2018).
11. Ma, M., Chen, X.-Y., Li, B. & Li, X.-T. Melatonin protects premature ovarian insufficiency induced by tripterygium glycosides: role of SIRT3. Am. J. Transl. Res. 9, 1580 (2017).
12. Morita, Y. et al. Oocyte apoptosis is suppressed by disruption of the acid sphingomyelinase gene or by sphingosine-1-phosphate therapy. Nat. Med. 6, 132–134 (2000).
13. Pascual, N. et al. Ceramide-1-phosphate has protective properties against cyclophosphamide-induced ovarian damage in a mouse model of premature ovarian failure. Hum. Reprod. 33, 844–859 (2018).
14. Roness, H., Kalich-Philosoph, L. & Meiraw, D. Prevention of chemotherapy-induced ovarian damage: possible roles for hormonal and non-hormonal attenuating agents. Hum. Reprod. Update 20, 759–771 (2014).
15. Chang, H.-M., Wu, H.-C., Sun, Z.-G., Lian, F. & Leung, P. C. Neurotrophins and glial cell line-derived neurotrophic factor in the ovary: physiological and pathophysiological implications. Hum. Reprod. Update 25, 224–242 (2019).
16. Dissen, G. A., Garcia-Rudaz, C. & Ojeda, S. R. Role of neurotrophic factors in early ovarian development. Semin. Reprod. Med. 27, 024–031 (2009).
17. Parees, A. et al. TrkB receptors are required for follicular growth and oocyte survival in the mammalian ovary. Dev. Biol. 267, 430–449 (2004).
18. Seifer, D. B., Fong, B., Shelden, R. M., Chen, S. & Dreyfus, C. F. Brain-derived neurotrophic factor: a novel human ovarian follicular protein. J. Clin. Endocrinol. Metab. 87, 655–659 (2002).
19. Harel, S. et al. Tyrosine kinase B receptor and its activated neurotrophins in ovaries from human fetuses and adults. Mol. Hum. Reprod. 12, 357–365 (2006).
20. D. A. S. M. & S. M. Brain-derived neurotrophic factor promotes bovine oocyte cytoplasmic competence for embryo development. Reproduction 129, 423–434 (2005).
21. Lee, E. et al. Beneficial effects of brain-derived neurotrophic factor on in vitro maturation of porcine oocytes. Reproduction 134, 405–414 (2007).
22. Ojeda, S. R., Roman, C., Tapia, V. & Dissen, G. A. Neurotrophic and cell-cell dependent control of early follicular development. Mol. Cell. Endocrinol. 163, 67–71 (2000).
23. Spears, N. et al. The role of neurotrophin receptors in female germ-cell survival in mouse and human. Development 130, 5481–5491 (2003).
24. Kerr, B., Garcia-Rudaz, C., Dorfman, M., Pareses, A. & Ojeda, S. R. TrkB and TrkB receptors facilitate follicle assembly and early follicular development in the mouse ovary. Reproduction 138, 131 (2009).
25. Czyzyk, A. et al. Brain-derived neurotrophic factor (BDNF) plasma concentration in patients diagnosed with premature ovarian insufficiency (POI). Gynecol. Endocrinol. 33, 143–147 (2017).
26. Johnson, J., Rose, R., Dworkin, M. & Ojeda, S. R. Prevention of chemotherapy-induced paracrine regulation of human oocyte maturation by BDNF and GDNF secreted by granulosa cells. Hum. Reprod. 26, 695–702 (2011).
27. Yu, Y. et al. Effects of combined epidural growth factor, brain-derived neurotrophic factor and insulin-like growth factor-1 on human oocyte maturation and early fertilized and cloned embryo development. Hum. Reprod. 27, 2146–2159 (2012).
28. Streiter, S., Fisch, B., Sabbah, B., Ao, A. & Abir, R. The importance of neuronal TrkB receptors for follicle assembly and early follicular development. Exp. Neurol. 141, 225–239 (1996).
29. Poduslo, J. F. & Curran, G. L. Permeability at the blood-brain and blood-nerve barriers of the neurotrophic factors: NGF, CNTF, NT-3, BDNF. Mol. Brain Res. 36, 280–286 (1996).
30. Morse, J. R. et al. Brain-derived neurotrophic factor (BDNF) prevents the degeneration of medial septal cholinergic neurons following fimbria transection. J. Neurosci. 13, 4146–4156 (1993).
31. Guo, W. et al. TrkB agonistic antibodies superior to BDNF: utility in treating motoneuron degeneration. Neurobiol. Dis. 132, 104590 (2019).
32. Meiraw, D. & Nugent, D. The effects of radiotherapy and chemotherapy on female reproduction. Hum. Reprod. Update 7, 535–543 (2001).
33. Kamravasman, S., Shaban, M. & Rahman, S. A. The prophylactic effect of Nigella sativa against cyclophosphamide in the ovarian follicles of matured adult mice: a preliminary study. J. Anim. Plant. Sci. 21, 88–91 (2014).
34. Wang, S. et al. Therapeutic potential of a TrkB agonistic antibody for Alzheimer’s disease. Theranostics 10, 6854–6874 (2020).
35. Meiraw, D. & Meiraw, D. Lewis, H., Nugent, D. & Epstein, M. Subclinical depletion of primordial follicular reserve in mice treated with cyclophosphamide: clinical importance and proposed accurate investigative tool. Hum. Reprod. 14, 1903–1907 (1999).
36. Nishi, Y. et al. Establishment and characterization of a steroidogenic human granulosa-like tumor cell line, KGN, that expresses functional follicle-stimulating hormone receptor. Endocrinology 142, 437–445 (2001).
37. V. M. A. C. M. A. Brain-derived neurotrophic factor promotes human granulosa-like tumor cell steroidogenesis and proliferation by activating the FSH receptor-mediated signaling pathway. Sci. Rep. 7, 1–13 (2017).
38. Meiraw, D., Lewis, H., Nugent, D. & Epstein, M. Subclinical depletion of primordial follicular reserve in mice treated with cyclophosphamide: clinical importance and proposed accurate investigative tool. Hum. Reprod. 14, 1903–1907 (1999).
39. Norm, S. D., Sarrel, P. M. & Nelson, L. M. Hormone replacement therapy in young women with primary ovarian insufficiency and early menopause. Fertil. Steril. 106, 1588–1599 (2016).
40. Narod, S. A. Hormone replacement therapy and the risk of breast cancer. Nat. Rev. Clin. Oncol. 8, 669 (2011).
41. M. J. S. M. C. Current status of hormone therapy and early menopause. J. Clin. Endocrinol. Metab. 94, 1129–1134 (2006).
42. Harel, S. et al. Tyrosine kinase B receptor and its activated neurotrophins in ovaries from human fetuses and adults. Mol. Hum. Reprod. 12, 357–365 (2006).
43. D. A. S. M. & S. M. Brain-derived neurotrophic factor promotes bovine oocyte cytoplasmic competence for embryo development. Reproduction 129, 423–434 (2005).
44. Lee, E. et al. Beneficial effects of brain-derived neurotrophic factor on in vitro maturation of porcine oocytes. Reproduction 134, 405–414 (2007).
45. Ojeda, S. R., Romero, C., Tapia, V. & Dissen, G. A. Neurotrophic and cell-cell dependent control of early follicular development. Mol. Cell. Endocrinol. 163, 67–71 (2000).
46. Spears, N. et al. The role of neurotrophin receptors in female germ-cell survival in mouse and human. Development 130, 5481–5491 (2003).
47. Kerr, B., Garcia-Rudaz, C., Dorfman, M., Pareses, A. & Ojeda, S. R. TrkB and TrkB receptors facilitate follicle assembly and early follicular development in the mouse ovary. Reproduction 138, 131 (2009).
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Author contributions

X.Q., W.G., and B.L. initiated the project and designed the study. X.Q. conducted the experiments and analyzed the data. Y.Z. contributed to the behavioral tests. Y.Z. and J.Q. provided the single-cell RNA-seq data of human ovarian follicles as well as experimental guidance on study design and safety evaluation. C.Y. provided the human ovarian samples. X.Q., B.L., and W.G. wrote, Y.Z, and J.Q edited the manuscript.

Competing interests

B.L. and W.G. are co-inventors of the filed patents related to the TrkB agonistic antibodies. B.L. is also a co-founder and Scientific Advisor of 4B Technologies, Limited, a biotech company that develops medicines for neurodegenerative diseases. The remaining authors declare no competing interests.

Additional information

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