Potential targets of transforming growth factor-beta1 during inhibition of oocyte maturation in zebrafish

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Abstract

Background: TGF-beta is a multifunctional growth factor involved in regulating a variety of cellular activities. Unlike mammals, the function of TGF-beta in the reproduction of lower vertebrates, such as fish, is not clear. Recently, we showed that TGF-beta1 inhibits gonadotropin- and 17alpha, 20beta-dihydroxyprogesterone (DHP)-induced maturation in zebrafish. The aim of the present study was to investigate the mechanisms underlying this action.

Method: To determine if the effect of TGF-beta1 on oocyte maturation involves transcription and/or translation, ovarian follicles were pre-treated with actinomycin D, a blocker of transcription, and cyclohexamide, an inhibitor of translation, and incubated with hCG or DHP, either alone or in combination with TGF-beta1 and oocyte maturation scored. To determine the effect of TGF-beta1 on mRNA levels of several key effectors of oocyte maturation, three sets of experiments were performed. First, follicles were treated with control medium or TGF-beta1 for 2, 6, 12, and 24 h. Second, follicles were treated with different concentrations of TGF-beta1 (0 to 10 ng/ml) for 18 h. Third, follicles were incubated with hCG in the absence or presence of TGF-beta1 for 18 h. At the end of each experiment, total RNA was extracted and reverse transcribed. PCR using primers specific for 20beta-hydroxysteroid dehydrogenase (20beta-HSD) which is involved in DHP production, follicle stimulating hormone receptor (FSHR), luteinizing hormone receptor (LHR), the two forms of membrane progestin receptor: mPR-alpha and mPR-beta, as well as GAPDH (control), were performed.

Results: Treatment with actinomycin D, a blocker of transcription, reduced the inhibitory effect of TGF-beta1 on DHP-induced oocyte maturation, indicating that the inhibitory action of TGF-beta1 is in part due to regulation of gene transcription. Treatment with TGF-beta1 caused a dose and time-dependent decrease in mRNA levels of 20beta-HSD, LHR and mPR-beta in follicles. On the other hand, TGF-beta1 had no effect on mPR-alpha mRNA expression and increased FSHR mRNA levels. Furthermore, hCG upregulated 20beta-HSD, LHR and mPR-beta mRNA levels, but this stimulatory effect was blocked by TGF-beta1.

Conclusion: These findings suggest that TGF-beta1 acts at multiple sites, including LHR, 20beta-HSD and mPR-beta, to inhibit zebrafish oocyte maturation.
Background
Transforming Growth Factor-β1 (TGF-β1) is the prototypical member of the TGF-β family [1,2]. Members of this family are implicated in diverse physiological processes, including reproduction. Three isoforms of TGF-β (TGF-β1, -β2, and -β3) are expressed in the mammalian ovary [2-4]. They have been shown to regulate follicle development, steroidogenesis, oocyte maturation, ovulation and follicular atresia [2-4]. There is molecular evidence for the presence of TGF-β1–3 in fish [5-7]. However, the role of TGF-β in fish reproduction is not well understood. Studies in zebrafish have suggested that TGF-β inhibits oocyte maturation [8]. In the goldfish, TGF-β has been reported to inhibit ovarian steroid production [9].

Ovarian development in fish is broadly divided into two major phases: growth and maturation. During oocyte growth, follicle stimulating hormone (FSH) stimulates production of estradiol-17β from the ovary. Estradiol-17β stimulates the production of vitellogenin by the liver. Vitellogenin is taken up by the developing oocyte and cleaved to yolk protein, which serves as a nutritional reserve for the developing embryo [8,10,11]. Oocyte maturation in teleosts is triggered by the release of leutinizing hormone (LH) from the pituitary. LH stimulates a number of signaling cascades culminating in the production of 17α-hydroxyprogesterone (17α-HP). In the granulosa cells, under the action of 20β-hydroxysteroid dehydrogenase (20β-HSD), 17α-HP is converted to 17α, 20β-dihydroxyprogesterone (DHP), the maturation inducing hormone (MIH) in cyprinids, such as zebrafish and goldfish. MIH activates the cytoplasmic maturation promoting factor (MPF), which is made up of two subunits: cyclin B (a regulatory subunit) and cdc2 (a catalytic subunit). MIH stimulates the de novo synthesis of cyclin B. Cyclin B protein binds to cdc2 to form MPF. The newly formed MPF is activated by phosphorylation of cdc2 on threonine 161. The active MPF, then, stimulates all the changes associated with oocyte maturation, such as germinal vesicle break down (GVBD), spindle formation, chromosome condensation and allows the transition from G2/M phase of meiosis [12-15].

Two isoforms of the MIH receptor, designated as membrane progestin receptor-α (mPR-α) and mPR-β, have recently been cloned in zebrafish [16]. Microinjection of zebrafish oocytes with antisense oligonucleotides to either mPR-α or mPR-β or both receptors has been shown to block MIH-induced maturation, indicating that both play a role in zebrafish oocyte maturation [17]. Originally discovered in sea-trout oocytes, several isoforms of mPR have also been discovered in humans and other vertebrates [16-20].

The zebrafish model has been used extensively for studies on early embryonic development. This model is also very useful for the investigation of ovarian follicle development and maturation because the zebrafish ovary contains ovarian follicles at different stages of development. We and others have been using zebrafish to examine the role of TGF-β superfamily in oocyte maturation [8,21-23]. Our previous work has shown that TGF-β1 inhibits human chorionic gonadotropin (hCG) and MIH-induced maturation in zebrafish [8]. We have also observed a decrease in TGF-β1 mRNA expression in maturing follicles. These findings suggest that TGF-β1 may play a role in preventing premature oocyte maturation in zebrafish. The present study is an attempt to elucidate the mechanisms underlying the inhibitory effect of TGF-β1 by identifying the potential target genes of TGF-β1. We report here our recent findings on the effect of TGF-β1 on the important effectors of oocyte maturation in zebrafish, including: 20β-HSD, LHR, mPR-α, and mPR-β.

Materials and methods
Animals
Adult zebrafish, Danio rerio, were purchased from a local pet supplier (Fish & Bird Emporium, Brampton, ON) and maintained in 10 L tanks of an AHAB System (Aquatic Habitats, FL) at 26 °C, under a 14-h light, 10-h dark photoperiod. The fish were fed twice daily with tropical fish food and supplemented with freshly hatched brine shrimp two or three times a week. Experiments were performed according to the Guide to the Care and Use of Experimental Animals published by the Canadian Council on Animal Care.

In vitro culture of zebrafish follicles
Female zebrafish that have a full-grown ovary were anesthetized using 3-aminobenzoic acid ethyl ester (Sigma-Aldrich Canada Inc., Oakville, ON) and decapitated. The ovaries were removed and follicles greater than 0.52 mm in diameter were collected, since previous studies in the zebrafish have shown that only follicles of this size can undergo maturation in vitro in response to hormones [21]. Approximately 20 follicles were placed into each well of a 24-well culture plate and pre-treated at 26 °C in either 1 ml of modified Cortland’s medium (MCM) or MCM + chemicals such as Actinomycin D or Cyclohexamide (Sigma-Aldrich, Mississauga, ON) for 2 hours. Pre-treated follicles were then incubated with the control medium, human recombinant TGF-β1 (R&D Systems, Minneapolis, MN), hCG (kindly provided by Dr. A. F. Parlow, National Hormone and Peptide Program, Torrance, CA), MIH (17α, 20β-DHP; Sigma-Aldrich Canada), either alone or in combination, as previously described [8]. Maturation was scored after 18 hours of incubation. Follicles that underwent germinal vesicle breakdown (GVBD) could be identified by their acquired translucency.
Total RNA extraction
Approximately 80 follicles per treatment group were used for RNA extraction. Total RNA was extracted using an RNeasy Mini kit (Qiagen Inc., Mississauga, ON) according to the manufacturer's suggested protocol. The RNase-free deoxyribonuclease Set (Qiagen) was also used during RNA isolation to remove any potential genomic DNA contamination.

Reverse Transcription (RT) and polymerase chain reaction (PCR)
Five micrograms of total RNA were reverse transcribed to cDNA at 37°C for 1.5 h in a total volume of 50 µl as previously described [21]. PCR was carried out in the presence of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.0 mM MgCl₂, 50 µM deoxynucleotide triphosphate, 1U Hotstar Taq (Qiagen) and 5 pmol primers. The primers for 20β-HSD, FSHR, LHR, mPR-α and mPR-β were designed according to the sequences from GenBank (Table 1). The semi-quantitative RT-PCR was validated by performing PCR reactions for different cycles to determine the cycle number that generated half maximal PCR product for each gene studied. PCR was carried out on an Eppendorf Master Cycler (Eppendorf AG, Hamburg, Germany) with the cycling profile: 20 s at 94°C, 30 s at 60–65°C (depending on the primer sets used), 30 s at 72°C followed by a 7-minute final extension at 72°C. The PCR products were electrophoresed on 1.5% agarose gels and visualized by ethidium bromide staining. The spot density of each PCR product was measured. For each gene studied, a cycle number within the exponential phase of the amplification curve was chosen for quantifying the expression of each gene in subsequent experiments. Accordingly, 20, 23, 30, 31, 33 and 32 cycles of PCR for GAPDH, 20β-HSD, FSHR, LHR, mPR-α and mPR-β, respectively, were selected as the optimal cycle numbers for measuring the levels of mRNA expression (Fig. 2).

Table 1: List of primers and their sequences

| Gene      | Sequence (5’-3’)                          | Accession # |
|-----------|-------------------------------------------|-------------|
| 20β-HSD   | Forward: TGC ACG AGT GGT CAA TGT GTC     | AF298898    |
|           | Reverse: ACT AGC TGT TCC GCA GGT TCC    |             |
| FSHR      | Forward: GGA TTC TTC ACC GTC TTC TCC    | AY278107    |
|           | Reverse: TGT AGC TGC TCA ACT CAA ACA    |             |
| LHR       | Forward: GGC GAA GCC TAG ATG GCA CAT    | AY424302    |
|           | Reverse: TCG CCA TCT GGT TCA ATA        |             |
| mPR-α     | Forward: CAG CGC CTA CTT CTC GT         | AY149121    |
|           | Reverse: CAC TGC ATC ATG AGC CAA AT     |             |
| mPR-β     | Forward: ACA ACG AGC TGC TGA ATG TG     | AY14920     |
|           | Reverse: ATG GCC CAG TTC AGA GTG AG     |             |

Statistical Analysis
All values are expressed as mean ± SEM of 3–4 replicates in one representative experiment. All experiments were performed three times to confirm the results using different batches of animals. To determine the statistical difference among different groups at the same time point, multiple group comparisons were performed by one-way ANOVA, followed by a Student-Newman-Keuls multiple group comparisons test, using the GraphPad InStat Software (GraphPad Inc., San Diego, CA). P < 0.05 was considered significant.

Results
Effects of transcriptional and translational inhibitors on final oocyte maturation
To determine if the effect of TGF-β on oocyte maturation requires transcription and/or translation, follicles were pretreated with cyclohexamide, an inhibitor of translation, or actinomycin D, an inhibitor of transcription. Pretreatment with cyclohexamide completely blocked hCG- and MIH-induced oocyte maturation. Pretreatment with actinomycin D blocked hCG-, but not MIH-, induced oocyte maturation (Fig. 1A). Interestingly, the inhibitory effect of TGF-β1 on MIH-induced maturation was partially reversed in the presence of actinomycin D (Fig. 1B), suggesting that the inhibitory effect of TGF-β1 on oocyte maturation involves in part transcriptional regulation.

Semi-quantitative RT-PCR Validation
Semi-quantitative RT-PCR assays were developed to examine the effect of TGF-β1 on the mRNA expression of 20β-HSD, FSHR, LHR, mPR-α and mPR-β. PCR assays were performed using ovarian cDNA as template for varying cycle numbers and spot densities of the resulting products were measured. A cycle number within the exponential phase of the amplification curve was chosen for quantifying the expression of each gene in subsequent experiments. Accordingly, 20, 23, 30, 31, 33 and 32 cycles of PCR for GAPDH, 20β-HSD, FSHR, LHR, mPR-α and mPR-β, respectively, were selected as the optimal cycle numbers for measuring the levels of mRNA expression (Fig. 2).
Figure 1
Effects of transcriptional and translational inhibitors on oocyte maturation. Ovarian follicles were pre-treated for 2 hours with 1 mg/ml Actinomycin D, Cyclohexamide or medium (untreated) and then incubated with: (A) Medium only (control), TGF-β1 (10 ng/ml), hCG (100 IU/ml), or a combination of hCG and TGF-β1 or (B) Medium only (control), TGF-β1 (10 ng/ml), MIH (100 ng/ml) or a combination of MIH and TGF-β1 for 18 hours. The rate of maturation was scored as percentage of follicles that underwent GVBD. Data represent the mean ± SEM of one representative experiment with four replicates. Different letters denote statistical significance (P < 0.05).
Figure 2
Validation of semi-quantitative RT-PCR for GAPDH (A), 20β-HSD (B), FSHR (C), LHR (D), mPR-α (E) and mPR-β (F). PCRs were performed using zebrafish ovarian cDNA as the template, amplified for varying cycle numbers and the density of the PCR products was quantified. Each value represents the mean ± SEM of three replicates in one representative RT-PCR. Representative ethidium bromide stained gel pictures were included. C = negative control; number on each lane represents the number of PCR cycles performed.
Effect of TGF-β1 on the mRNA expression of 20β-Hydroxysteroid dehydrogenase

To test whether TGF-β1 affects the mRNA expression of 20β-HSD, a time course study was performed where follicles were treated with 10 ng/ml of TGF-β1 for 2, 6, 12 and 24 hours. A set of untreated follicles was included for each time point as controls. Total RNA was extracted and RT-PCRs were performed. A time-dependant decrease in 20β-HSD mRNA expression relative to control levels was found in TGF-β1 treated follicles. A significant inhibitory effect was observed at 24 hours of treatment (Fig. 3A). A dose-response study was performed where follicles were treated with increasing doses of TGF-β1 (0, 0.1, 1 and 10 ng/ml) for 18 hours. Total RNA was extracted from each set of treated follicles and subjected to RT-PCR analyses. A dose-dependant decrease in 20β-HSD expression in response to TGF-β1 treatment was found (Fig. 3B). The strongest inhibition was seen upon treatment with 10 ng/ml of TGF-β1. Finally, the combinational effect of hCG and TGF-β1 on 20β-HSD mRNA expression was tested. Follicles were incubated with control medium, hCG (100 IU/ml), TGF-β1 (10 ng/ml), or hCG plus TGF-β1, for 18 hours. Treatment with hCG resulted in an increase in 20β-HSD mRNA levels. However, treatment with TGF-β1 decreased basal and hCG-induced 20β-HSD mRNA levels (Fig. 3C).

Effect of TGF-β1 on the mRNA expression of follicle stimulating hormone receptor

Treatment with TGF-β1 resulted in a significant increase in FSHR mRNA levels from 6 to 18 hours after treatment, with the maximal stimulation at 12 hours post treatment (Fig. 4A, B). The stimulatory effect of TGF-β1 on FSHR mRNA expression was observed for all doses tested (Fig. 4B). Treatment with hCG had no significant effect on basal FSHR mRNA expression when compared to the control (Fig. 4C). Similarly, hCG did not affect TGF-β1-induced FSHR mRNA levels (Fig. 4C).

Effect of TGF-β1 on the mRNA expression of the luteinizing hormone receptor

TGF-β1 significantly decreased LHR mRNA expression at 18 and 24 hours after treatment, but had no effect at 2 to 12 hours post treatment (Fig. 5A, B). At 18 hours after treatment, TGF-β1 inhibited LHR mRNA expression in a dose-dependant manner (Fig. 5B). Treatment with hCG increased LHR mRNA levels. However, when TGF-β1 was added together with hCG, the stimulatory effect of hCG on LHR mRNA expression was blocked (Fig. 5C).

Effect of TGF-β1 on the mRNA expression of membrane progesterin receptors

No significant effect of TGF-β1 on mPR-α mRNA expression was observed. The time course study showed no significant difference in the mRNA expression of mPR-α at
TGF-β1 stimulates FSHR mRNA expression. (A) Follicles were incubated with control medium or 10 ng/ml of TGF-β1 for 2, 6, 12 and 24 hours. (B) Follicles were treated with different concentrations (0, 0.1, 1 and 10 ng/ml) of TGF-β1 for 18 hours. (C) Follicles were treated with medium (control), hCG (100 IU/ml), TGF-β1 (10 ng/ml), or a combination of hCG and TGF-β1 for 18 hours. At the end of each incubation, total RNA was extracted and reverse transcribed. PCR was carried out using primers for FSHR and GAPDH. Each value represents the mean ± SEM of three replicates in one representative RT-PCR. Statistical significance (P < 0.05) is indicated by either an * or a different letter. The insets show the representative ethidium bromide stained gels.

Figure 4
TGF-β1 suppresses LHR mRNA expression. Follicles were treated with (A) 10 ng/ml of TGF-β1 for 2, 6, 12 and 24 hours; (B) 0.1, 1, or 10 ng/ml of TGF-β1 for 18 hours; and (C) hCG (100 IU/ml), TGF-β1 (10 ng/ml), either alone or in combination, for 18 hours. Each value represents the mean ± SEM of three replicates in one representative RT-PCR. Different letters denote statistical significance (P < 0.05). The insets show the original ethidium bromide stained gels.

Figure 5
TGF-β1 has no effect on mPR-α mRNA expression. Follicles were incubated with (A) 10 ng/ml of TGF-β1 for 2, 6, 12 and 24 hours; (B) different concentrations of TGF-β1 for 18 hours; and (C) medium only (control), hCG (100 IU/ml), TGF-β1 (10 ng/ml) or hCG + TGF-β1 for 18 hours. Each value represents the mean ± SEM of three replicates in one representative RT-PCR. The insets show representative ethidium bromide stained gels. Neither hCG nor TGF-β1 had an effect on mPR-α mRNA expression.

Figure 6
TGF-β1 downregulates mPR-β mRNA expression. (A) Time course study of the effect of TGF-β1 on mPR-β mRNA expression. Follicles were treated with 10 ng/ml of TGF-β1 for 2, 6, 12 and 24 hours. (B) Follicles were treated with different concentrations (0, 0.1, 1 and 10 ng/ml) of TGF-β1 for 18 hours. (C) Follicles were incubated with medium (control), hCG (100 IU/ml), TGF-β1 (10 ng/ml) or hCG + TGF-β1 for 18 hours. Each value represents the mean ± SEM of three replicates in one representative RT-PCR. Different letters or "*" denote statistical significance (*P < 0.05). The insets show the representative ethidium bromide stained gels.
any of the time points examined (Fig. 6A). Similarly, treatment with different concentrations of TGF-β1 for 18 hours did not result in a significant change in mPR-α mRNA levels (Fig. 6B). Neither hCG nor TGF-β1 had a significant effect on mPR-α mRNA expression (Fig. 6C). In contrast to mPR-β, similar treatment with TGF-β1 resulted in a dose- and time-dependent inhibition of mPR-β mRNA levels. The inhibitory effect was observed at 18 and 24 hours after treatment and all doses of TGF-β1 tested caused a significant decrease in mPR-β mRNA levels (Fig. 7A, B). Incubation of follicles with hCG resulted in a strong stimulation of mPR-β mRNA expression. The stimulatory effect of hCG on mPR-β mRNA expression was partially blocked by TGF-β1 (Fig. 7C).

Discussion

Currently, little is known about the role of TGF-β in regulating ovarian functions in lower vertebrates, such as fish. Recent studies have suggested that TGF-β inhibits steroid production in the goldfish ovary [9] and oocyte maturation in zebrafish [8]. In the present study, we further examined the cellular mechanisms underlying the inhibitory effect of TGF-β in oocyte maturation. We have shown that TGF-β1 inhibits mRNA expression of 20β-HSD, the key enzyme involved in MIH production, as well as LHR and mPR-β. These novel findings suggest that TGF-β1 inhibits multiple targets in the oocyte maturation pathway, both upstream and downstream of MIH.

One of the potential targets of TGF-β1 identified in this study is 20β-HSD. We found that TGF-β1 inhibited basal and hCG-induced 20β-HSD mRNA levels. TGF-β1 alone induced a dose- and time-dependent inhibitory effect on 20β-HSD mRNA expression. Treatment with hCG increased 20β-HSD mRNA levels; however, in the presence of TGF-β1, the effect of hCG was blocked. 20β-HSD activity in the ovary and its stimulation by gonadotropins and cAMP-enhancing drugs has been demonstrated in many species [10,11,24-28]. Our finding that hCG stimulates 20β-HSD is consistent with studies in mammals [24,28] and a recent study in Nile Tilapia, which reported an increase in the mRNA expression of 20β-HSD in response to hCG treatment [26]. The observation of decreased 20β-HSD mRNA levels after TGF-β1 treatment suggests that TGF-β1 may inhibit 20β-HSD activity, leading to a decrease in MIH production. This notion is supported by a recent report that TGF-β1 inhibits the conversion of 17α-HP to DHP in the goldfish [9]. It is possible that one of the actions of TGF-β1 is to decrease MIH production, and thus inhibit oocyte maturation.

In this study, we observed that hCG increased LHR but had no effect on FSHR mRNA levels. Several studies conducted in fish on the effect of gonadotropin on LHR and FSHR expression have yielded inconsistent results. In African and Channel catfish, it has been reported that hCG treatment caused an activation of the LHR and cAMP mediated pathways, as well as a slight increase in FSHR mRNA levels [29-31]. However, a two-fold increase in FSHR expression, but no change in LHR expression in pre-maturational follicles of rainbow trout in response to treatment with partially purified gonadotropins has also been reported [32]. The reason for such discrepancy is unclear, however, it could be due to species specificity or variation in treatment conditions.

An interesting finding from this study is that TGF-β1 differentially regulates FSHR and LHR mRNA levels. We observed that basal FSH receptor mRNA levels were increased, while basal- and hCG-induced LH receptor mRNA levels were decreased, upon incubation of follicles with TGF-β1. TGF-β1 has been reported to regulate LH and FSH binding sites in mammals, however, whether TGF-β1 has a stimulatory or inhibitory effect appears to be species-dependent. TGF-β1 decreased basal and FSH-induced LH binding sites in porcine granulosa cells but enhanced FSH-induced LH binding in rat granulosa cells [33]. FSH induced FSH binding in porcine granulosa cells and this effect was attenuated by TGF-β1. On the other hand, FSH decreased its own binding levels in rat granulosa cells and this effect was also blocked by TGF-β1 [33]. Our finding that TGF-β1 increased FSHR mRNA levels is consistent with previous studies in rat granulosa cells [34,35]. However, our observation that TGF-β1 decreased LHR mRNA levels is opposite to studies in rat [36] and chicken [37] granulosa cells. In these studies, it was reported that TGF-β1 inhibited LHR mRNA expression. Whether this is due to species variation as in the case of rat and pig or to the difference in follicle development between fish and higher vertebrates awaits more studies in fish species. The finding that TGF-β1 inhibited LHR mRNA expression suggests that the inhibitory effect of TGF-β1 on hCG-induced oocyte maturation may be due, in part, to the downregulation of LH receptor by TGF-β1. Since FSH is known to be a major regulator of oocyte growth [10,11], by stimulating FSH receptor expression TGF-β1 may play a role in promoting follicle growth.

These findings, together with our previous observation that TGF-β1 mRNA levels are higher in growing follicles than in maturing follicles, suggest that TGF-β1 may stimulate follicle development and inhibit precocious oocyte maturation in the zebrafish ovary. This possibility will be investigated further in the future.

A recent study has shown that microinjection of zebrafish oocytes with antisense oligonucleotides to either mPR-α or mPR-β or both causes similar marked decreases in the rates of oocyte maturation, suggesting that both subtypes are obligatory for oocyte maturation in zebrafish [17]. In this study, we observed a strong induction of mPR-β.
mRNA levels by hCG and an inhibitory effect of TGF-β1 on both basal and hCG-induced mPR-β mRNA expression. These findings support the role of mPR-β in oocyte maturation and suggest that one of the mechanisms by which TGF-β1 inhibits hCG- and MIH-induced oocyte maturation is by the downregulation of MIH receptors, specifically mPR-β. However, we found that neither hCG nor TGF-β1 had an effect of mPR-α mRNA levels, suggesting that the two membrane progestin receptors are under differential regulation. It has been reported that hCG caused an increase in the mPR protein expression in sea-trout oocytes [18]. The sea-trout mPR has a higher homology to mPR-α (80%) than to mPR-β (46%). It remains to be determined if hCG regulates mPR-α and mPR-β protein levels in the zebrafish. Recently, Kazeta et al. (2005) reported that hCG did not change mRNA levels of mPR-α and mPR-β at 5 and 10 h after hCG treatment [38]. The difference between this and our studies may be due to the duration of hCG treatment as 18 h was used in our study.

Conclusion

Based on our findings that TGF-β1 downregulates basal and hCG-induced LHR, 20β-HSD, and mPR-β mRNA levels, we propose that TGF-β1 acts upon multiple targets to exert its inhibitory effect on oocyte maturation. TGF-β1 may downregulate LHR, leading to decreased signal transduction and decreased production of 17α-hydroxyprogesterone. TGF-β1 may inhibit basal and gonadotropin-induced MIH production by inhibiting 20β-HSD. Finally, TGF-β1 may inhibit the expression of the MIH receptor, such as mPR-β, on the oocyte surfaces, leading to a reduction of MPF activation and subsequent oocyte maturation. This model can be tested in the future by examining the protein levels on these molecules once antibodies become available. Similarly, the physiological significance of TGF-β on oocyte maturation will be confirmed using loss-of-function approaches.

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References

1. Massague J: TGF-beta signal transduction. Annu Rev Biochem 1998, 67:753-791.
2. Peng C: The TGF-beta superfamily and its roles in the human ovary and placentia. J Obstet Gynaecol Can 2003, 25:834-844.
3. Knight PG, Glister C: Local roles of TGF-beta superfamily members in the control of ovarian follicle development. Anim Reprod Sci 2003, 78:165-183.
4. Ingman WV, Robertson SA: Defining the actions of transforming growth factor beta in reproduction. Bioessays 2002, 24:904-914.
5. Hardie LJ, Laing KJ, Daniels GD, Grabowski PS, Cunningham C, Secombes CJ: Isolation of the first piscine transforming growth factor beta gene: analysis reveals tissue specific expression and a potential regulatory sequence in rainbow trout (Oncorhynchus mykiss). Cytokine 1998, 10:555-563.
6. Yin Z, Kwang J: Molecular isolation and characterization of transforming growth factor beta 1 from activated leucocytes. Fish Shellfish Immunol 2000, 10:309-318.
7. Harms CA, Kennedy-Stoskopf S, Horne WA, Fuller FJ, Tompkins WA: Cloning and sequencing hybrid striped bass (Morone saxatilis x M. chrysops) transforming growth factor-beta (TGF-beta), and development of a reverse transcription quantitative competitive polymerase chain reaction (RT-qPCR) assay to measure TGF-beta mRNA of teleost fish. Fish Shellfish Immunol 2000, 10:61-85.
8. Kohli G, Hu S, Clelland E, Di Muccio T, Rodenstein J, Peng C: Cloning of Transforming Growth Factor-Beta-1 (TGF-β1) and Its Type II receptor from Zebrasfish Ovary and Role of TGF-β1 in Oocyte Maturation. Endocrinology 2003, 144.
9. Calp MK, Matsumoto JA, Van Der Kraak G: Activin and transforming growth factor–beta signaling of ovarian steroidogenesis in the goldfish. Gen Comp Endocrinol 2003, 132:142-150.
10. Nagahama Y, Yoshikuni M, Yamashita M, Tokumoto T, Katsu Y: Regulation of oocyte growth and maturation in fish. Curr Top Dev Biol 1995, 30:103-145.
11. Nagahama Y: 17 alpha, 20 beta-dihydroxy-4-pregnene-3-one, a maturation-inducing hormone in fish oocytes: mechanisms of synthesis and action. Steroids 1997, 62:190-196.
12. Kajura-Kobayashi H, Yoshida N, Sagata N, Yamashita M, Nagahama Y: The Mos/MAPK pathway is involved in metaphase II arrest as a cyostatic factor but is neither necessary nor sufficient for initiating oocyte maturation in goldfish. Dev Genes Evol 2000, 210:416-425.
13. Katsu Y, Yamashita M, Nagahama Y: Translational regulation of cyclin B mRNA by 17 alpha, 20 beta-dihydroxy-4-pregnene-3-one (maturation-inducing hormone) during oocyte maturation in a teleost fish, the goldfish (Carassius auratus). Mol Cell Endocrinol 1999, 158:79-85.
14. Kanoto T, Yanagawa T, Yoshida N, Yamashita M: Introduction of cyclin B induces activation of the maturation-promoting factor and breakdown of germinal vesicle in growing zebrafish oocytes unresponsive to the maturation-inducing hormone. Dev Biol 1997, 190:142-152.
15. Kanoto T, Kanada T, Yamashita M: Dispersion of cyclin B mRNA aggregation is coupled with translational activation of the mRNA during zebrafish oocyte maturation. Dev Biol 2001, 229:421-431.
16. Zhu Y, Bond J, Thomas P: Identification, classification, and partial characterization of genes in humans and other vertebrates homologous to a fish membrane progestin receptor. Proc Natl Acad Sci U S A 2003, 100:2237-2242.
17. Thomas P, Peng Y, Zhu Y, Detwiler C, Doughty K: Multiple rapid progesterin actions and progesterin membrane receptor subtypes in fish. Steroids 2004, 69:567-573.
18. Zhu Y, Rice CD, Peng Y, Pace M, Thomas P: Cloning, expression, and characterization of a membrane progestin receptor and evidence it is an intermediary in meiotic maturation of fish oocytes. Proc Natl Acad Sci U S A 2003, 100:2231-2236.
19. Hammes SR, Niumura S, Kawakami SY, Senthilkumaran B, Sudhakumar C, Chang XT, Kobayashi T, Oba Y, Guan G, Yoshiaura Y, Yoshikuni M, Nagahama Y, Kazeto Y, Adachi S, Yamauchi K: Steroids and oocyte maturation—a new look at an old story. Mol Endocrinol 2004, 18:769-775.
20. Kazeto Y, Goto-Kazeto R, Thomas P, Trant JM: Molecular characterization of three forms of putative membrane-bound progestin receptors and their tissue-distribution in channel catfish, Ictalurus punctatus. J Mol Endocrinol 2005, 34:781-791.
21. Peng Y, Patel H, Mukai S, Melino C, Garg R, Ni X, Chang J, Peng C: Activin, inhibin, and follistatin in zebrafish ovary: expression and role in oocyte maturation. Biol Reprod 2000, 62:1585-1592.
22. Peng Y, Ge W: Gonadotropin and activin enhance maturational competence of oocytes in the zebrafish (Danio rerio). Biol Reprod 2002, 66:259-265.
23. Peng Y, Ge W: Epidermal Growth factor and TGF-α promote zebrafish oocyte maturation in vitro: potential role of the ovarian activin regulatory system. Endocrinology 2002, 143:47-54.
24. Niimura S, Kawakami SY: Changes in the activities of hydroxysteroid dehydrogenases in mouse oocytes during meiotic maturation. J Reprod Dev 2003, 49:451-456.

25. Kazeto Y, Adachi S, Yamauchi K: 20beta-Hydroxysteroid dehydrogenase of the Japanese eel ovary: its cellular localization and changes in the enzymatic activity during sexual maturation. Gen Comp Endocrinol 2001, 122:109-115.

26. Senthilkumaran B, Sudhakumari CC, Chang XT, Kobayashi T, Oba Y, Guan G, Yoshiura Y, Yoshikuni M, Nagahama Y, Kazeto Y, Adachi S, Yamauchi K: Ovarian carbonyl reductase-like 20beta-hydroxysteroid dehydrogenase shows distinct surge in messenger RNA expression during natural and gonadotropin-induced meiotic maturation in Nile tilapia. Biol Reprod 2002, 67:1080-1086.

27. Nagahama Y KHYG: Stimulation of 17,20-dihydroxy-4-pregn-3-en-3-one production in the granulosa cells of amago salmon, Oncorhynchus rhodurus, by cyclic nucleotides. J Exp Zool 1985, 236:371-375.

28. Stocco CO, Chedrese J, Deis RP: Luteal expression of cytochrome P450 side-chain cleavage, steroidogenic acute regulatory protein, 3beta-hydroxysteroid dehydrogenase, and 20alpha-hydroxysteroid dehydrogenase genes in late pregnant rats: effect of luteinizing hormone and RU486. Biol Reprod 2001, 65:1114-1119.

29. Vischer HF, Bogerd J: Cloning and functional characterization of a gonadal luteinizing hormone receptor complementary DNA from the African catfish (Clarias gariepinus). Biol Reprod 2003, 68:262-271.

30. Kumar RS, Ijiri S, Trant JM: Molecular biology of the channel catfish gonadotropin receptors: 2. Complementary DNA cloning, functional expression, and seasonal gene expression of the follicle-stimulating hormone receptor. Biol Reprod 2001, 65:710-717.

31. Kumar RS, Ijiri S, Trant JM: Molecular biology of channel catfish gonadotropin receptors: 1. Cloning of a functional luteinizing hormone receptor and preovulatory induction of gene expression. Biol Reprod 2001, 64:1010-1018.

32. Bobe J, Maugars G, Nguyen T, Rime H, Jalabert B: Rainbow trout follicular maturational competence acquisition is associated with an increased expression of follicle stimulating hormone receptor and insulin-like growth factor 2 messenger RNAs. Mol Reprod Dev 2003, 66:46-53.

33. Gitay-Goren H, Kim IC, Miggans ST, Schomberg DW: Transforming growth factor beta modulates gonadotropin receptor expression in porcine and rat granulosa cells differently. Biol Reprod 1993, 48:1284-1289.

34. Dunkel L, Tilly JL, Shikone T, Nishikori K, Hsueh AT: Follicle-stimulating hormone receptor expression in the rat ovary: increases during prepubertal development and the regulation by the opposing actions of transforming growth factors and alpha. Biol Reprod 1994, 50:940-948.

35. Inoue K, Nakamura K, Abe K, Hirakawa T, Tsuchiya M, Oomori Y, Matsuda H, Miyamoto K, Minegishi T: Mechanisms of action of transforming growth factor beta on the expression of follicle-stimulating hormone receptor messenger ribonucleic acid levels in rat granulosa cells. Biol Reprod 2003, 69:1238-1244.

36. Inoue K, Nakamura K, Abe K, Hirakawa T, Tsuchiya M, Matsuda H, Miyamoto K, Minegishi T: Effect of transforming growth factor beta on the expression of luteinizing hormone receptor in cultured rat granulosa cells. Biol Reprod 2002, 67:610-615.

37. Johnson AL, Bridgham JT, Woods DC: Cellular mechanisms and modulation of activin A- and transforming growth factor beta-mediated differentiation in cultured hen granulosa cells. Biol Reprod 2004, 71:1844-1851.

38. Kazeto Y, Goto-Kazeto R, Trant JM: Membrane-bound progesterone receptors in channel catfish and zebrafish ovary: changes in gene expression associated with the reproductive cycles and hormonal reagents. Gen Camp Endocrinol 2005, 142:204-211.