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14. ABSTRACT: Transforming growth factor beta (TGFB) signaling regulates fundamental reproductive events via TGFB receptor complexes (TGFBR1/TGFBR2) and downstream SMAD proteins. To determine potential effect of overactivation of TGFB signaling in distinct cellular compartments, we generated a mouse model containing a constitutively active TGFBR1 using growth differentiation factor 9 (Gdf9)-Cre (termed TGFBR1-gCA). We showed that sustained activation of TGFBR1 disrupts folliculogenesis via affecting ovarian reserve and follicle growth/development. Ovarian tumor tissues from TGFBR1-gCA mice were positive for granulosa cell markers. RNA-Seq analysis using ovarian RNA from TGFBR1-gCA mice and controls identified a number of genes associated with folliculogenesis, oogenesis, proliferation, and differentiation. Histological and molecular analyses provided evidence of overactivation of TGFB signaling in ovarian granulosa cell compartment. The mouse model may be further exploited to define the cellular and molecular mechanisms of TGFB/activin downstream signaling in granulosa cell tumor development.

15. SUBJECT TERMS
TGF-beta signaling, granulosa cell tumors, pathogenesis, mouse model, follicular development, RNA-Seq, ovarian tumor

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1. Introduction

Transforming growth factor beta (TGFB) family members regulate a myriad of cellular functions and key reproductive processes in a contextually dependent manner via transmembrane receptors (TGFBR1/TGFBR2) and intracellular mediators of SMAD proteins. TGFB signaling is indispensable for reproductive development and disease (Li 2014). The function of TGFB signaling in the ovary, particularly in the oocyte is not clear. The aim of this project is to define the oncogenic role and associated mechanisms of constitutively active TGFBR1 in our mouse model in which constitutive activation of TGFBR1 was driven by *Gdf9*-iCre. To complete the aim, we used both in vivo and in vitro approaches. These studies provided new insights into ovarian granulosa cell tumor development.

2. Keywords

TGF-beta signaling, granulosa cell tumors, pathogenesis, mouse model, follicular development, RNA-Seq, ovarian tumor

3. Accomplishments

- *What were the major goals of the project?*

The major goals of the project are:

(1) Define the oncogenic role of constitutively active TGFBR1 in the oocyte using mice
Major Task 1: Define the oncogenic role of constitutively active TGFBR1 in the oocyte
Major Task 2: Deconstruct the role of sustained activation of oocyte TGFBR1 in ovarian tumor development using an in vitro approach

(2) Identify the oncogenic insult of sustained activation of TGFB signaling in the oocyte
Major Task 3: Explore the stage-specific function of constitutively active TGFBR1 in the oocyte
Major Task 4: Discover novel regulatory mechanisms of granulosa cell proliferation resulting from constitutive activation of TGFBR1 in the oocyte

- *What was accomplished under these goals?*

1.) Major activities and specific objectives

Major Task 1: Define the oncogenic role of constitutively active TGFBR1 in the oocyte

We completed proposed subtasks 1-6. In brief, we obtained local IACUC approval and ACURO approval of the AUP. We performed studies to determine primordial follicle activation, cell apoptosis, and performed immunohistochemical analysis of ovaries from control and TGFBR1-gCA mice using multiple granulosa cell markers.

Major Task 2: Deconstruct the role of sustained activation of oocyte TGFBR1 in ovarian tumor development using an in vitro approach

Under the proposed subtasks, we collected ovaries and isolated oocyte cumulus complexes from control and TGFBR1-gCA mice to determine the role of sustained activation of TGFBR1.

Major Task 3: Explore the stage-specific function of constitutively active TGFBR1 in the oocyte

Under the proposed Major Task 3, we completed proposed subtasks and generated *TGFBR1 Zp3*-Cre constitutively active mice (TGFBR1-zCA) and characterized these mice using histological, immunohistochemical, and apoptotic analyses.

Major Task 4: Discover novel regulatory mechanisms of granulosa cell proliferation resulting from constitutive activation of TGFBR1 in the oocyte

Under the proposed Major Task 4, we have prepared ovarian RNA samples, performed RNA-seq, and analyzed RNA-seq results. In the no-cost extension period, we focused on data mining and validation to achieve the milestone of defining key regulators/pathways for sex cord-stromal tumors.

2.) Significant results or key outcomes

Major Task 1: Define the oncogenic role of constitutively active TGFBR1 in the oocyte

(1) Overactivation of TGFBR1 using *Gdf9*-Cre perturbed follicular development at an early stage. Because *Gdf9*-Cre is expressed in primordial follicles, we performed histological analysis of follicular development in control and TGFBR1-gCA mice at early postnatal stages. To perform follicle counting, ovaries were first serially sectioned (5 μm) and stained with periodic acid Schiff's (PAS) and hematoxylin. Follicles were counted from every 5th section. Follicle classification was based on morphological criteria (Myers *et al.* 2004, Bristol-Gould *et al.* 2006).

Follicle quantification did not show a significant difference in primordial follicles between control and TGFBR1-gCA mice at postnatal day 5 (P5), although there was a reduction of primary follicle numbers in the

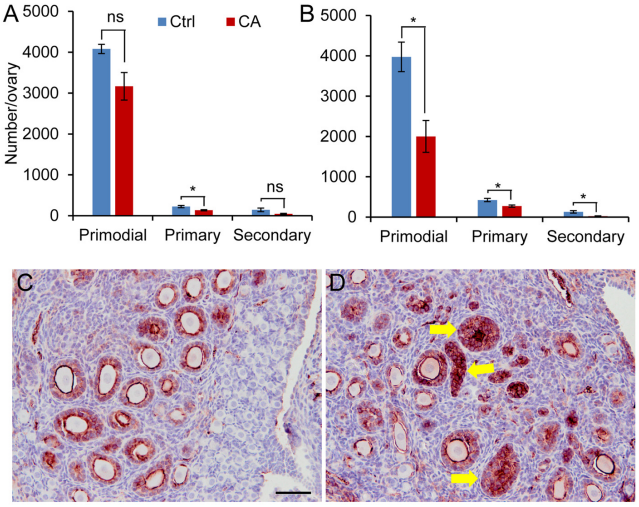

Figure 1. Altered follicular development in TGFBR1-gCA mice. (**A** & **B**) Follicle counts of control and TGFBR1-gCA ovaries at P5 (A) and P7 (B). Follicles were counted from every 5th section. Data are mean ± s.e.m. n = 3. $^{*}P < 0.05$. ns, not significant. (**C** & **D**) Immunohistochemical localization of INHA in P7 control (C) and TGFBR1-gCA (D) ovaries. Arrows (D) indicate abnormal follicle structures. Experiment was performed using ABC method, and signals were developed using NovaRED Peroxidase Substrate Kit. Magnification: 200x.

TGFBR1-gCA mice versus controls (Figure 1A). At P7, the number of primordial follicles, primary follicles, and secondary follicles was reduced in TGFBR1-gCA ovaries compared with controls (Figure 1B). At this stage, abnormal follicle structures were detected in the ovary of TGFBR1-gCA mice, evidenced by INHA staining (Figure 1C and D). These results suggest that sustained activation of TGFBR1 in the oocytes using *Gdf9*-Cre disrupts ovarian folliculogenesis by affecting ovarian reserve and subsequent follicle development.

(2) Activation of TGFBR1 using *Gdf9*-Cre did not promote apoptosis in the oocyte. It has been suggested that loss of oocytes during follicular development may alter the differentiation and cell fate of ovarian granulosa cells (Pitman *et al.* 2012). We therefore performed apoptosis analysis to determine whether there is a link between oocyte apoptosis and abnormal granulosa cell differentiation and tumor formation. To achieve this goal, we utilized a commercially available *In situ* Apoptosis Detection Kit to stain ovarian samples at P3, P7, P12, and P21. To validate this kit, we showed that samples treated with DNase I contained abundant apoptotic cells (i.e., positive control), whereas negative controls where TdT was substituted with water showed only background staining (Figure 2A and B). Our apoptosis analysis using ovarian samples from several critical time points during early follicular development did not reveal alteration of oocyte apoptosis in TGFBR1-gCA ovaries (Figure 2C and D). Extensive apoptosis was detected in ovarian somatic cells at P21 in TGFBR1-gCA mice compared with controls, where apoptosis was confined to certain follicles that were atretic or would potentially be atretic (Figure 2E and F). Thus, these results suggest that oocyte apoptosis may not be a main contributing factor to ovarian tumor development in our mouse model.

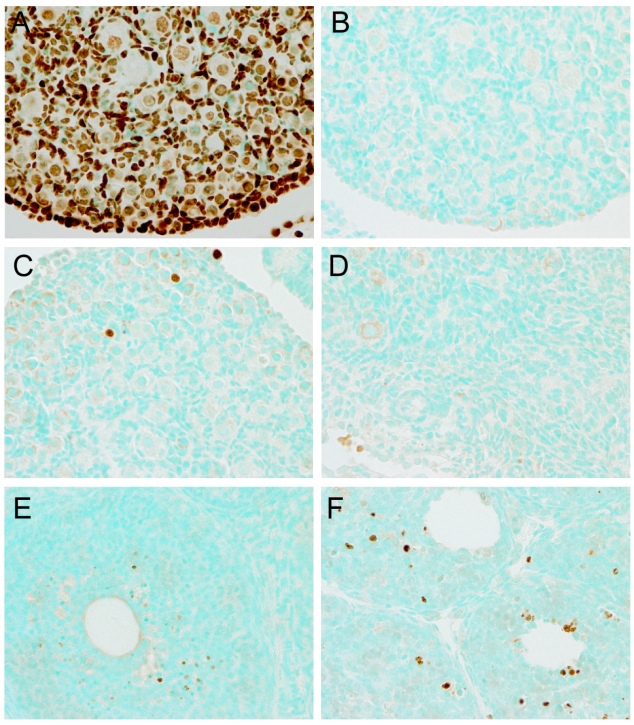

Figure 2. Apoptosis analysis of postnatal ovaries from control and TGFBR1-gCA mice. (**A**) P3 ovarian sections treated with DNase I as positive control. (**B**) Negative control where TdT was replaced with water. (**C-F**) Representative images of apoptosis analysis of P3 and P21 control (C and E) and TGFBR1-gCA (D and F) ovarian sections. Apoptotic cells were labeled with terminal deoxynucleotidyl transferase (TdT) and signals were detected using DAB. Sections were counterstained with Methyl Green. At least 4 independent samples per group were used in this analysis. Magnification: 400x.

(3) Molecular analysis of ovarian tumor type. To define the molecular identity of ovarian tumors in TGFBR1-gCA mice, we performed immunostaining to determine expression of a granulosa cell lineage maker FOXL2 (Schmidt *et al.* 2004) and 3 other granulosa cell-expressed proteins, FOXO1, INHA, and AMH. DDX4, a germ cell marker, was also included. Briefly, tissue processing and embedding were carried out using the histology core facility of the Department of Veterinary Integrative Biosciences at Texas A&M University. Paraffin sections (5

μm) were used for both immunofluorescence and immunohistochemistry as described (Li *et al.* 2011, Gao *et al.* 2014).

The localization of FOXL2 (Figure 3A), INHA (Figure 3C), FOXO1 (Figure 3E), AMH (Figure 3G), and DDX4 (Figure 3I) was detected in the granulosa cell or oocyte compartment of the control mice, while ovarian tumor tissues from TGFBR1-gCA mice were immunoreactive with FOXL2 (Figure 3B), INHA (Figure 3D), and FOXO1 (Figure 3F), supporting the development of granulosa cell tumors in these mice. However, expression of AMH was close to background level in the tumor tissues (Figure 3H). These tumors did not express DDX4 (Figure 3J). Representative negative controls using rabbit and goat IgGs were respectively shown in Figure 3K and L. Because granulosa cell tumors can express Sertoli cell marker (Liu *et al.* 2015), we examined whether Sertoli cell-like components were present in the TGFBR1-gCA tumors.

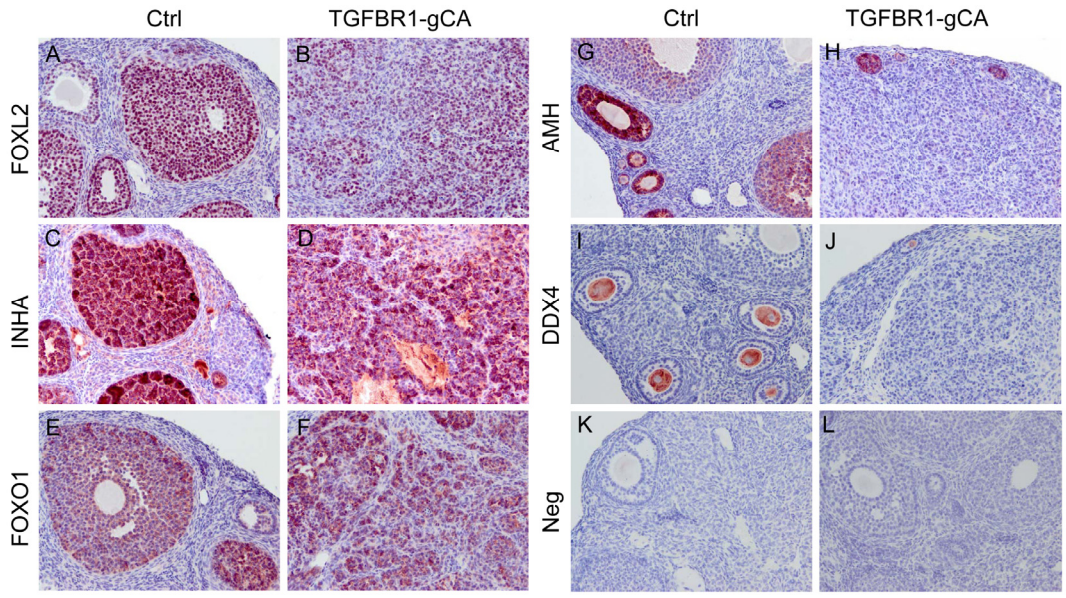

Figure 3. Identification of tumor type using molecular markers. (**A-J**) Expression of granulosa cell and germ cell markers in control and TGFBR1-gCA ovaries. Representative images from immunohistochemical analysis of FOXL2 (A and B), INHA (C and D), FOXO1 (E and F), AMH (G and H), and DDX4 (I and J) are shown. (**K** & **L**) Negative controls using isotype-matched rabbit (K) and goat (L) IgGs. Mice at the age of 8 weeks were utilized for this experiment. Experiment was performed using ABC method, and signals were developed using NovaRED Peroxidase Substrate Kit. n = 5. Magnification: 200x.

Major Task 2: Deconstruct the role of sustained activation of oocyte TGFBR1 in ovarian tumor development using an in vitro approach

We took advantage of a small molecule inhibitor of TGFBR1 to examine the oncogenic property of the constitutively active TGFBR1 using *in vitro* culture approach. In brief, we collected ovaries from control and TGFBR1-gCA mice and cultured them using Transwell membrane insert (Dutta *et al.* 2014). Ovaries were cultured in the presence or absence of SB-505124 for 10 days at 37°C supplemented with 5% CO2. Culture was fed every 2 days. After that, the ovaries were fixed and processed for histological analysis. We also isolated oocyte-granulosa cell complex (OGC) by using 0.1% collagenase digestion of P12 ovaries (Eppig & O'Brien 1996).

While OGCs retrieved from the control mice were morphologically normal and similar in size, OGCs obtained from the TGFBR1-gCA mice varied in size and contained large abnormal structures, suggesting that overactivation of TGFBR1 using *Gdf9*-Cre promotes the formation of abnormal OGCs. Interestingly, our culture experiment revealed disorganized and abnormal follicle structures within vehicle-treated TGFBR1-gCA ovaries (Figure 4A and B). Incubation of TGFBR1-gCA ovaries with SB-505124 (10 µM) seemed to improve follicle development in the TGFBR1-gCA ovaries (Figure 4C and D). Therefore, these studies reinforce that ovarian tumor development in TGFBR1-gCA mice is due to enhanced TGFBR1 activity.

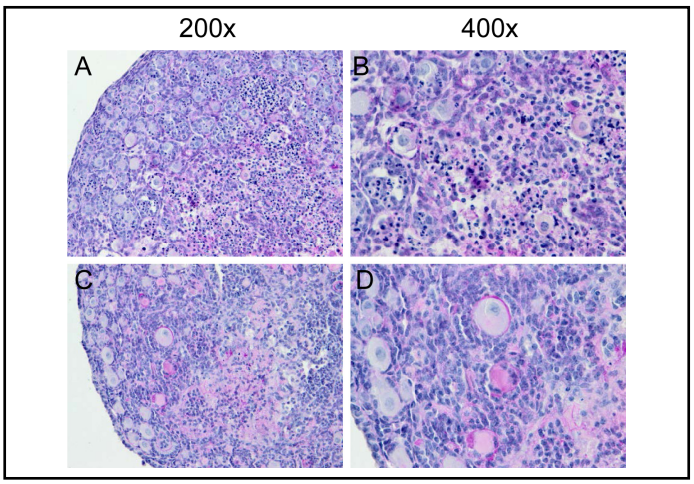

Figure 4. Histological analysis of cultured TGFBR1-gCA ovaries treated with vehicle and TGFBR1 inhibitor. (**A** & **B**) TGFBR1-gCA ovaries treated with vehicle control. (**C** & **D**) TGFBR1-gCA ovaries treated with SB-505124. Ovary culture was performed using Transwell membrane insert in the presence or absence of SB-505124 (10 µM) for 10 days before histological analysis using periodic acid Schiff's (PAS) and hematoxylin staining. Magnification: 200x (A & C) and 400x (B & D).

Major Task 3: Explore the stage-specific function of constitutively active TGFBR1 in the oocyte

Gdf9-iCre is expressed in the germ cell from the primordial follicle stage (Lan *et al.* 2004). *Zp3*-Cre, which is expressed in growing oocytes, but not non-growing oocytes of primordial follicles (de Vries *et al.* 2000). To complement our TGFBR1-gCA mice, we generated a mouse model using *Zp3*-Cre line, which was purchased from The Jackson Laboratory (Stock no. 003651) (de Vries *et al.* 2000). First, we crossed the *Zp3*-Cre mice with *TGFBR1*$^{\text{CA Lox/Lox}}$ mice to obtain *TGFBR1*$^{\text{CA Lox/+}}$; *Zp3*-Cre male mice. Then these mice were bred with *TGFBR1*$^{\text{CA Lox/Lox}}$ females to generate the *TGFBR1*$^{\text{CA Lox/Lox}}$; *Zp3*-Cre female mice (termed TGFBR1-zCA). In contrast to the TGFBR1-gCA mice, no ovarian abnormality was found macroscopically (not shown). To examine whether molecular changes occur in the TGFBR1-zCA mice, we performed immunohistochemical analysis using control and TGFBR1-zCA ovaries and antibodies directed to INHA, FOXL2, and DDX4. As expected, granulosa cells from control mice were positively stained for INHA and FOXL2 (Figure 5A, B, E, and F; next page), while oocytes were immunoreactive with anti-DDX4 antibody (Figure 5I and J). In line with the normal histological observations in the TGFBR1-zCA ovaries, no differences in the expression pattern of INHA, FOXL2, and DDX4 were found in the ovaries between TGFBR1-zCA mice (Figure 5C, D, G, H, K, and L) and control mice (Figure 5A, B, E, F, I, and J).

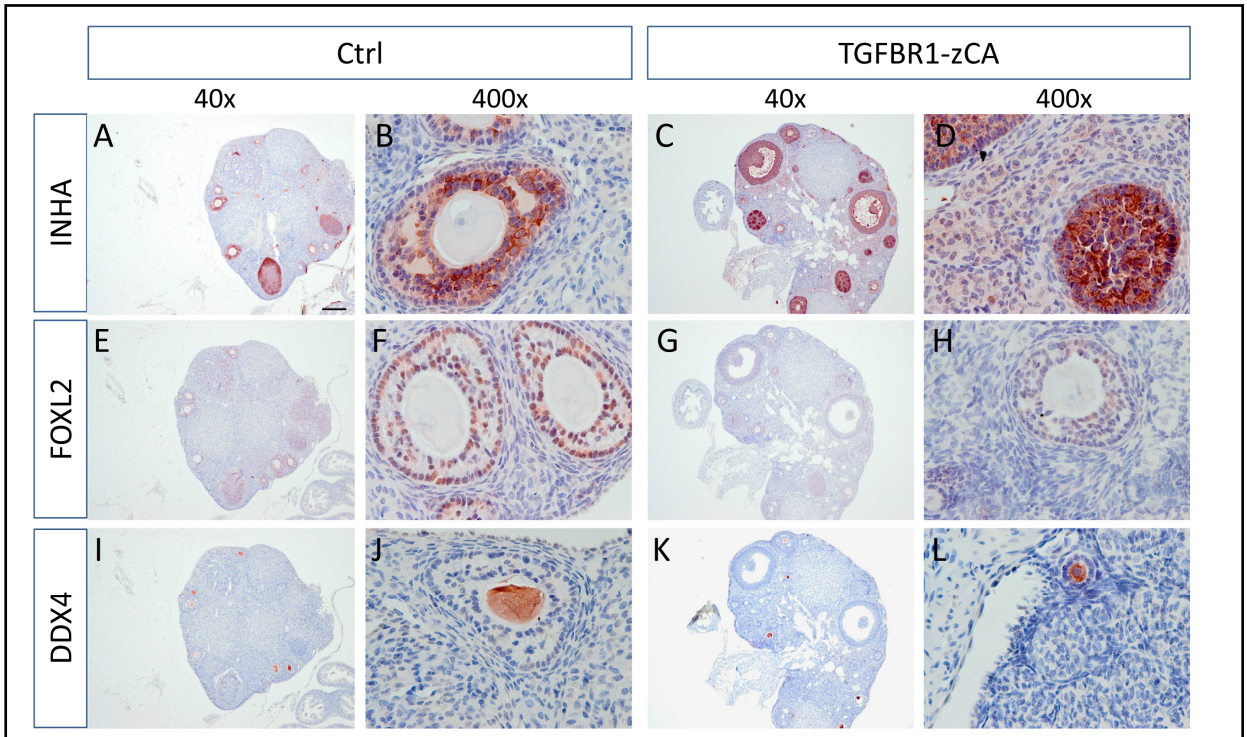

Figure 5. Immunohistochemical analysis of ovaries from TGFBR1-zCA mice. (**A-L**) Immunolocalization of granulosa cell and germ cell markers in control and TGFBR1-zCA ovaries. Panels (B, F, J, D, H, L) are higher magnification images for the corresponding panes (A, E, I, C, G, K). Note a similar expression pattern of INHA (A-D), FOXL2 (E-H), and DDX4 (I-L) between control and TGFBR1-zCA mice. Immunohistochemical analysis was performed using mice at the age of 8 weeks. Experiments were conducted using ABC method, along with NovaRED Peroxidase Substrate Kit. n = 5. Scale bar is representatively shown in (A) and equals 20 μm (B, F, J, D, H, L) and 200 μm (A, E, I, C, G, K).

The finding that TGFBR1-zCA did not develop ovarian tumor phenotype posed several possibilities including potential requirement of activation of TGFBR1 during primordial stage of follicle development for ovarian tumorigenesis, direct or indirect activation of TGFBR1 within somatic cell compartment in TGFBR1-gCA ovaries, and/or low efficiency of TGFBR1 activation in TGFBR1-zCA ovaries. Our qPCR analysis using total RNAs prepared from TGFBR1-zCA and controls did not show increased expression of *TGFBR1*^{CA} transcripts in TGFBR1-zCA ovaries (not shown), which is in line with the incapability of detecting recombined conditional beta-catenin in the ovary using *Zp3*-Cre line potentially due to the low ratio of the oocytes to somatic tissue in the ovary (de Vries *et al.* 2000).

Major Task 4: Discover novel regulatory mechanisms of granulosa cell proliferation resulting from constitutive activation of TGFBR1 in the oocyte.

RNA-seq is a powerful tool with high sensitivity and resolution to profile gene expression. To identify novel regulators/pathways of ovarian granulosa cell tumors, we isolated total RNA from TGFBR1-gCA and control mice at the age of 7 days and performed RNA-seq analysis. The assay and data processing were performed using Texas A&M Institute for Genomic Sciences and

Society (TIGSS). The volcano plot and heat map of the top 50 expressed genes were shown (Figure 6). Initial analysis has identified 1301 genes that were differentially regulated. An Ingenuity Pathway Analysis identified a number of genes that are associated with folliculogenesis and oogenesis (Figure 7).

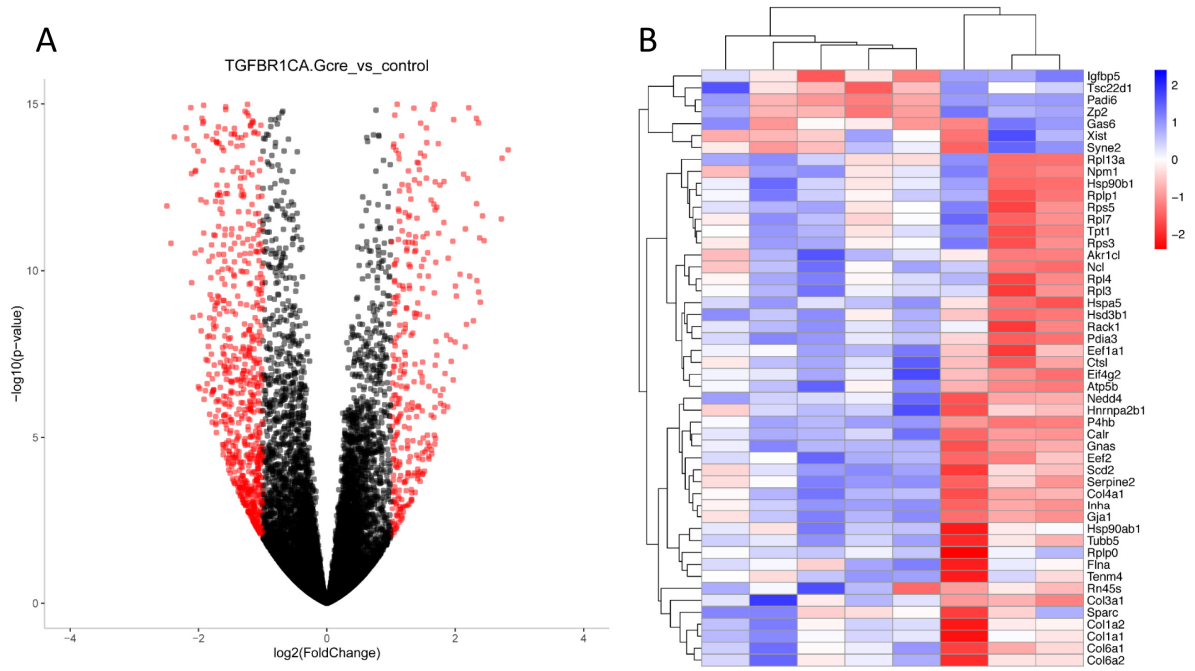

Figure 6. RNA-seq analysis of ovarian samples from control and TGFBR1-gCA mice. (**A**) Volcano plot of genes expressed in control and TGFBR1-gCA ovaries. (**B**) Heat map showing the top 50 expressed genes in 7-day-old control and TGFBR1-gCA ovaries. n = 4. RNA-seq was conducted using the Texas A&M Institute for Genomic Sciences and Society (TIGSS).

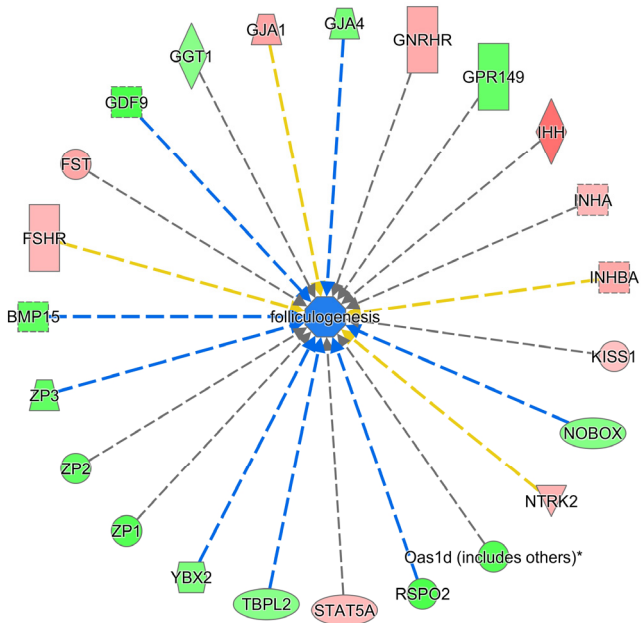

Figure 7. Ingenuity pathway analysis identifies dysregulated genes associated with folliculogenesis. A preliminary Ingenuity Pathway Analysis (IPA) showed that a number of critical genes that are involved in folliculogenesis and/or oogenesis were dysregulated in TGFBR1-gCA ovaries at the age of 7 days.

To validate the RNA-Seq results, we performed real-time PCR analysis of candidate genes. Our results showed significantly reduced expression of mRNA transcripts for Y box protein 2 (*Ybx2*; Figure 8A), cytoplasmic polyadenylation element binding protein 1 (*Cpeb1*; Figure 8B), G-protein coupled receptor 3 (*Gpr3*; Figure 8C), NOBOX oogenesis homeobox (*Nobox*; Figure 8E), and SEBOX homeobox (*Sebox*; Figure 8F). Significance was not found in *Hormad1* expression due to the large variation of the gene expression level in control group (Figure 8D).

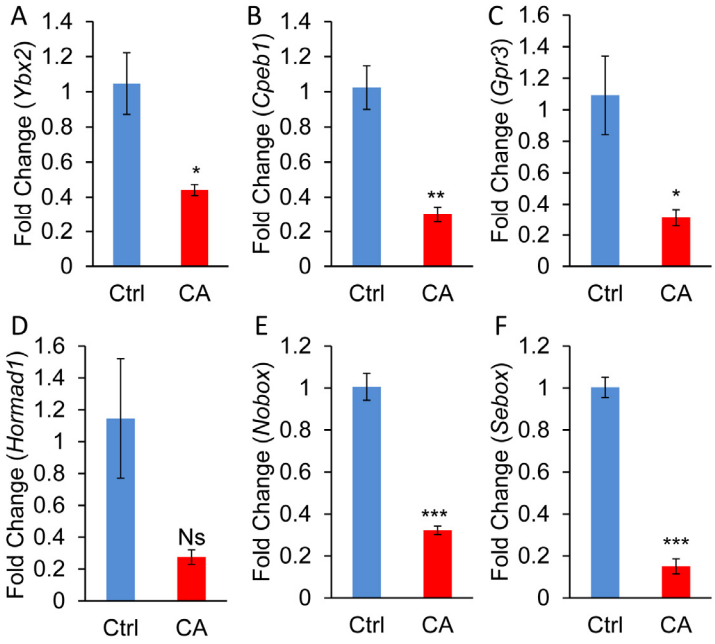

Figure 8. Validation of differentially regulated genes revealed by RNA-Seq in TGFBR1-CA^{G9Cre} ovaries. (**A-F**) Real-time PCR analysis of expression of *Ybx2*, *Cpeb1*, *Gpr3*, *Hormad1*, *Nobox*, and *Sebox* in ovaries from control and TGFBR1-CA^{G9Cre} mice at postnatal day 7 (PD7). Real-time PCR was performed using ΔΔCT method. Data are mean ± s.e.m. $n = 4$. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. Ns, not significant.

Real-time PCR was performed to examine overactivation of TGFBR1 in ovarian tissues from TGFBR1-CA^{G9Cre} mice. *TGFBR1*^{CA} transcripts were highly expressed in the ovaries of TGFBR1-CA^{G9Cre} mice (Figure 9A; top of next page), coinciding with increased expression of *Smad7*, a TGFB target gene (Figure 9B). Moreover, the mRNA expression of *Inha* was increased and *Zp3* reduced at PD7 (Figure 9C and D), corroborating altered granulosa cell and oocyte properties.

Of note, pyruvate dehydrogenase kinase 4 (PDK4) may serve as a metabolic regulator of cancer development (Liu *et al.* 2014). Interestingly, *Pdk4* has been identified as a significantly upregulated gene in TGFBR1-CA^{G9Cre} ovaries in our RNA-Seq analysis. The result was confirmed by real-time PCR (Figure 10A; next page). To determine the localization of *Pdk4* in the ovary, we performed a pilot RNAscope assay. RNAscope 2.5 HD Reagent Kit and *Pdk4* probe (Catalog no. 437161) were obtained from Advanced Cell Diagnostics. Expression of *Pdk4* was detectable in granulosa cells (Figure 10B) and abnormal follicle structures (Figure 10C). Positive control peptidylprolyl isomerase B (Ppib; Figure 10D) and negative control dihydrodipicolinate reductase (DapB; Figure 10E) were included.

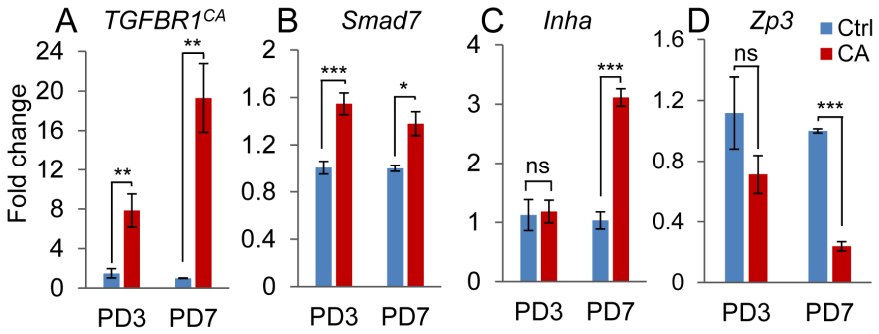

Figure 9. Evidence of TGFBR1 activation in ovarian granulosa cells of TGFBR1-CA^{G9Cre} mice. (**A-D**) Real-time PCR analysis of expression of *TGFBR1*^{CA}, *Smad7*, *Inha*, and *Zp3* in ovaries from control and TGFBR1-CA^{G9Cre} mice at PD3 and PD7. Real-time PCR was performed using ΔΔCT method. Data are mean ± s.e.m. n = 4-5. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. Ns, not significant.

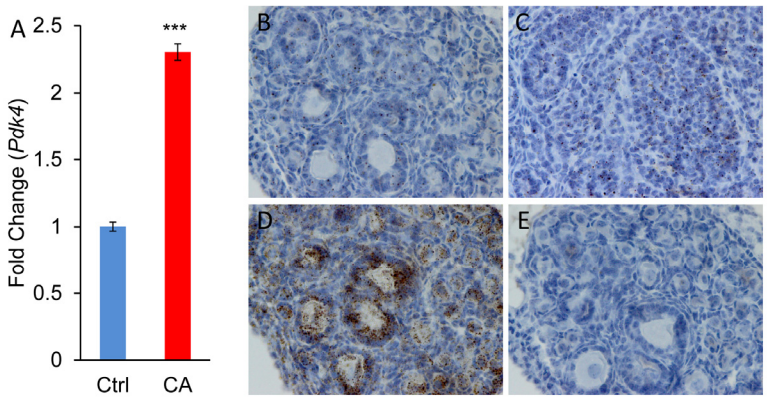

Figure 10. Expression and localization of PDK4 in mouse ovary. (A) Real-time PCR analysis of expression of *Pdk4* in ovaries from control and TGFBR1-CA^{G9Cre} mice at PD7. Data are mean ± s.e.m. n = 4. $^{***}P < 0.001$. (**B-E**) RNAscope *in situ* hybridization analysis of *Pdk4* mRNA distribution using PD7 control (B, D, E) and TGFBR1-CA^{G9Cre} ovaries (C). Positive and negative controls were shown in (D) and (E), respectively.

- What opportunities for training and professional development has the project provided?

  Nothing to Report.
- How were the results disseminated to communities of interest?

  Nothing to Report.
- What do you plan to do during the next reporting period to accomplish the goals?

  Project is completed this year.

4. Impact

- What was the impact on the development of the principal discipline(s) of the project?

Our studies showed the importance of dysregulated TGFB signaling in the pathogenesis of ovarian granulosa cell tumors. Potential impact of this project is to identify new targets for the treatment of ovarian granulosa cell tumors.

- What was the impact on other disciplines?

  Nothing to Report.

- What was the impact on technology transfer?

  Nothing to Report.

- What was the impact on society beyond science and technology?

  Nothing to Report.

5. Changes/Problems

- Changes in approach and reasons for change

  Nothing to Report.

- Actual or anticipated problems or delays and actions or plans to resolve them

  Nothing to Report.

- Changes that had a significant impact on expenditures

  Nothing to Report.

- Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

  Nothing to Report.

6. Products

- Publications, conference papers, and presentations

We published two peer-reviewed papers in *Biol Reprod* and *Reprod Biol Endocrinol*. We presented 2 posters: one at an international meeting [i.e. the Society for the Study of Reproduction (SSR)] and the other at a local meeting (*Texas Forum for Reproductive Sciences*).

Peer-reviewed Publication:

(1) Fang X, Gao Y, **Li Q**. SMAD3 Activation: A Converging Point of Dysregulated TGF-Beta Superfamily Signaling and Genetic Aberrations in Granulosa Cell Tumor Development? *Biology of reproduction*. 2016 95(5):105.

(2) Gao Y, Fang X, Vincent DF, Threadgill DW, Bartholin L, **Li Q**. Disruption of postnatal folliculogenesis and development of ovarian tumor in a mouse model with aberrant transforming growth factor beta signaling. ***Reprod Biol Endocrinol***. 2017 Dec 8;15(1):94. doi: 10.1186/s12958-017-0312-z.

Conference and Presentations

(1) Gao Y and **Li Q**. Sustained activation of transforming growth factor beta signaling using growth differentiation factor 9-driven iCre disrupts postnatal folliculogenesis and promotes malignant transformation of ovarian somatic cells (**Poster presentation**). *22nd Annual Meeting, Texas Forum for Reproductive Sciences*, Houston, Texas, USA, April 21 & 22, 2016.

(2) Fang X, Gao Y, Konganti K, Ni N, Threadgill D, Burghardt R, Bartholin L, and **Li Q**. Disruption of folliculogenesis and development of ovarian tumors in a mouse model with aberrant TGFB receptor-mediated signaling (**Poster presentation**). *49th Annual Meeting, Society for the Study of Reproduction*, Washington DC, USA, July 13–16, 2017.

- Website(s) or other Internet site(s)

  Nothing to Report.
- Technologies or techniques

  Nothing to Report.
- Inventions, patent applications, and/or licenses

  Nothing to Report.
- Other Products

  Nothing to Report.

7. Participants & Other Collaborating Organizations

- What individuals have worked on the project?

Name	Project Role	Nearest person month worked each year	Contribution to the project
Yang Gao	Graduate Student	6 Calendar (Year 1-2) 3 Calendar (Year 3)	Mouse breeding, immunostaining, apoptosis detection, RNAscope, data analysis, genotyping and histological analysis, co-author of 2 meeting abstracts and 2 publications
Xin Fang	Graduate student	3 Calendar (Year 2) 6 Calendar (Year 3)	Genotyping and histological analysis, presenter of a meeting abstract, co-author of 2 publications
Qinglei Li	PI	3 Calendar (Year 1-3)	Project supervision and data analysis, co-author of 2 meeting abstracts, corresponding author of 2 publications
David Threadgill	Co-investigator	0.6 Calendar (Year 1-3)	Experimental design, data analysis, co-author of a meeting abstract and a research paper
Robert Burghardt	Co-investigator	0.6 Calendar (Year 1-3)	Contributed to ovarian tissue culture, supervised project graduate student (YG), co-author of a meeting abstract

- Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

(1) 1 R01 HD087236-01A1 03/01/2017-02/28/2022

TGF-beta signaling in endometrial cell function and dysfunction

National Institutes of Health, NICHD

The major goal of this project is to identify the mechanisms whereby TGFβ signaling regulates endometrial cell function and determine how abnormal TGFβ signaling causes endometrial dysfunction and fertility problems.

Role: PI

(2) Title: EZH2 and endometrial cancer. 2018-2020

Funding agency: Texas A&M University funds-T3 grant

Goal: the goal of this project is to determine the role of EZH2 in the development of endometrial cancer.

Role: PI

- What other organizations were involved as partners?

  Nothing to report.

8. Special Reporting Requirements

None.

9. Appendices

References Cited under *introduction* and *significant results or key outcomes*

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