

## Application of the Dual-Luciferase Reporter Assay to the Analysis of Mouse Kit and Yy1 Promoter Activation Regulation by Sohlh1

<sup>1,3</sup>Shengyang Li, <sup>1</sup>Bing Du, <sup>3</sup>Guangbin Luo, <sup>2</sup>Wei Yan and <sup>1</sup>Zhihong Zheng

<sup>1</sup>Department of Reproductive Biology and Transgenic Animals,  
China Medical University, 110001 Shenyang, P.R. China

<sup>2</sup>Department of Physiology and Cell Biology,

University of Nevada School of Medicine, NV 89557 Reno, USA

<sup>3</sup>College of Animal Science and Veterinary Medicine, Shenyang Agricultural University,  
110866 Shenyang, P.R. China

**Abstract:** Kit is significantly downregulated in Sohlh1 deletion ovaries and Sohlh1 was over-expressed in Yy1 cKO ovaries and whether Sohlh1 regulates kit and Yy1 transcription is unknown. Here, researchers examine the promoter transcription activation of Kit and Yy1 regulated by Sohlh1 using dual-luciferase reporter assays. The Kit responsiveness of the *Sohlh1* gene has been validated and provided evidence of Sohlh1 transcriptional activation of the *Kit* gene promoter in the mouse ovary. In contrast, Sohlh1 has little effect on the essential activity of Yy1 promoter. Researchers identified -1769 to -297 as the transcriptional activation region of Yy1 promoter and revealed strong inhibitor in -297 to +135 region where maybe G-quadruplex motifs exist. Researchers found two Yy1 binding Sites in Sohlh1 promoter and surmise that Yy1 negatively regulates Sohlh1 in mouse ovary.

**Key words:** Mouse, Sohlh1, Kit, Yy1, dual-luciferase reporter assay, China

---

### INTRODUCTION

Mouse are endowed with a large complement of primordial follicles within their ovaries at birth. Some primordial follicles are recruited to grow into primary and larger follicles but most oocytes die by apoptosis during primordial follicle assembly (Brown *et al.*, 2010; Xu *et al.*, 2011). Several transcription factors and signaling molecules such as Sohlh1 (spermatogenesis and oogenesis-specific bHLH transcription factor 1), Sohlh2 (spermatogenesis and oogenesis-specific bHLH transcription factor 2, Figla (factor in the germ line, alpha), *Nobox* (newborn ovary homeobox gene), *Lhx8* (Lim homeobox gene 8), Yy1 (Yin-Yang1), Kit and its Ligand (KitL) have been implicated in primordial to primary and growing follicle transition (De Felici, 2000; Rajkovic *et al.*, 2004; Hutt *et al.*, 2006; Pangas *et al.*, 2006; Joshi *et al.*, 2007; Choi *et al.*, 2008a, b; Griffith *et al.*, 2011).

Sohlh1 is preferentially expressed in germ cells of the embryonic ovary and in oocytes of primordial and primary follicles. Sohlh1 homozygous null females were infertile with atrophic ovaries that lacked oocytes at 10 weeks and

occupied  $\approx 1/10$  of the WT volume. There appears to be a defect in follicle development during the primordial to primary follicle transition in Sohlh1 deletion ovaries (Pangas *et al.*, 2006).

During early oocyte and follicular development, paracrine communications between oocytes and surrounding granulosa cells are critical for normal cell development and function (Gilchrist *et al.*, 2004). KitL localized to granulosa cells is a growth factor that exerts an influence on oocytes through binding its tyrosine kinase receptor, Kit (localized to oocytes) (Hutt *et al.*, 2006). Kit-KitL interactions play an anti-apoptotic effect in fetal oogonia and oocytes and improve oocytes of primordial to progress beyond the primary follicle in postnatal ovaries (Driancourt *et al.*, 2000).

Yy1 is a multifaceted polycomb group gene that activates, represses or initiates transcription of a diverse assortment of genes (Hinkins *et al.*, 2005). Recent study had presented that YY1 function in the oocyte is critically required for oocyte growth and granulosa cell expansion (Griffith *et al.*, 2011). Deletion of Yy1 from oocytes of primary follicles and growing follicles results in a blockage of folliculogenesis. Yy1 cKO mice are infertile and their

ovaries lack mature follicles due to defects in proliferation and expansion of granulosa cells (Griffith *et al.*, 2011). Several germ cell specific genes are down regulated in Yy1 cKO ovaries such as Growth differentiation factor 9 (Gdf9) and Lhx8 which are also down regulated in Sohlhl1 deletion ovaries (Jagarlamudi and Rajkovic, 2011).

Kit is significantly downregulated in Sohlhl1 deletion ovaries and Sohlhl1 was over-expressed in Yy1 cKO ovaries (Choi *et al.*, 2008a, b; Griffith *et al.*, 2011). Interestingly when Kit, Yy1 or Sohlhl1 was absent there was much similarity in oogenesis and ovary clinical phenotype such as sterile, atrophic ovaries, postnatal oocyte loss and the blockage of early folliculogenesis (Hutt *et al.*, 2006; Pangas *et al.*, 2006; Griffith *et al.*, 2011). Researchers suppose the relationship of gene regulation may exist among the three genes. In early mouse folliculogenesis whether Sohlhl1 regulates Kit and Yy1 transcription is unknown. Here, researchers examine the promoter transcription activation of Kit and Yy1 regulated by Sohlhl1 using dual-luciferase reporter assays.

**MATERIALS AND METHODS**

All mouse experiments were carried out on a C57BL/6 background.

**Mouse Kit and Yy1 promoter-luciferase reporter constructs:** Plasmid pGL3-basic consisting of a multiple cloning site in front of the luciferase reporter gene was used to create Kit and Yy1 promoter-luciferase reporter constructs. PCR was performed using mouse tail DNA as template and Platinum® Pfx DNA Polymerase (Invitrogen). Primers were designed that would amplify the region from -2298 to +124 of the *Kit* gene (GenBank Accession Number: NC\_000071.5): KitF 5' CGGGGTACCCAACC CAAGAGGAAAGTGTAGC3' and KitR 5' CCCAAGCTT

CCACGGAGCAGGACCAA 3' (KpnI site is underlined and HindIII site is in bold), thermal cycling conditions consisted of an initial denaturation step at 94°C for 4 min followed by 30 cycles of 94°C for 20 sec, 60°C for 30 sec and 68°C for 2 min 30 sec final extension was 68°C for 10 min and -2202 to +135 of the *Yy1* gene (GenBank Accession Number: NC\_000078.5): Yy1F 5' CGGGGTACC AGCCTTCCCACATTTCATAC3' and Yy1R 5' TCCCCCGG GCGTGGCGATGTA GAGGGTGT 3' (KpnI site is underlined and SmaI site is italicised), thermal cycling conditions consisted of an initial denaturation step at 94°C for 4 min followed by 30 cycles of 94 for 20 sec, 58°C for 30 sec and 68°C for 2 min 30 sec, final extension was 68°C for 10 min. KpnI and HindIII double digestions were carried out on the Kit promoter and pGL3-basic vector. Subcloning into pGL3-Basic placed the Kit (-2298 to +124) promoter fragments upstream of the promoter-less luciferase gene for promoter activity analysis. The successfully constructed plasmid were named pGL3-Kit-A. The method of the construction of pGL3-Yy1-A was identical to that of pGL3-Kit-A, only used KpnI and SmaI instead. AxyPrep™ Plasmid Miniprep Kit was used to purify the plasmid from Escherichia coli DH5α.

To generate of Kit and Yy1 promoter deletion constructs, the design of primers used for PCR amplifying different lengths of promoters was based on rightly sequenced pGL3-Kit-A and pGL3-Yy1-A as the templates (Table 1). There were difficulties with the SmaI digest hence HindIII was used instead to generate Yy1 promoter deletion constructs. The identities of all Kit and Yy1 promoter constructs were verified by restriction mapping and nucleotide sequencing.

**Construction of expression vector for mouse Sohlhl1:** Total RNA was isolated from new born WT mouse ovaries by using TRIzol (Invitrogen). RNA

Table 1: Oligonucleotides used for PCR to generate Kit and Yy1 promoters deletion constructs

Primer name	Sequence	Products size/bp	Reaction condition
pGL3-Kit-B	F:5'-CGGGGTACCATGATACTCAATGAGGCCATG-3' R:5'-CCCAAGCTT CCACGGAGCAGGACCAA-3'	2054 (-1930 to +124)	94°C 20 sec, 60°C 30 sec, 68°C 2 min 20 sec 30 cycles
pGL3-Kit-C	F:5'-CGGGGTACCGACTTAAAGAACCCCAAAA-3' R:5'-CCCAAGCTT CCACGGAGCAGGACCAA-3'	1543 (-1419 to +124)	94°C 20 sec, 60°C 30 sec, 68°C 1 min 40 sec 30 cycles
pGL3-Kit-D	F:5'-CGGGGTACCATCTTGCTCCTAAGGCAGACC-3' R:5'-CCCAAGCTT CCACGGAGCAGGACCAA-3'	1002 (-878 to +124)	94°C 20 sec, 60°C 30 sec, 68°C 1 min 10 sec 30 cycles
pGL3-Kit-E	F:5'-CGGGGTACCCCGTCTCTCTACCAAC-3' R:5'-CCCAAGCTT CCACGGAGCAGGACCAA-3'	640 (-516 to +124)	94°C 20 sec, 60°C 30 sec, 68°C 50 sec 30 cycles
pGL3-Kit-F	F:5'-CGGGGTACCTGTCTTAGAGGGCACAGCG-3' R:5'-CCCAAGCTT CCACGGAGCAGGACCAA-3'	308 (-184 to +124)	94°C 20 sec, 60°C 30 sec, 68°C 25 sec 30 cycles
pGL3-Yy1-B	F:5'-CGGGGTACC GTAGTTAACCTAGAAACGAGG-3' R: 5'-CCCAAGCTTCGTGGCGATGTAGAGGGTGT-3'	1904 (-1769 to +135)	94°C 20 sec, 58°C 30 sec, 68°C 2 min 30 cycles
pGL3-Yy1-C	F:5'-GGGTACCTGTACTTATAATAGGAAATC-3' R: 5'-CCCAAGCTTCGTGGCGATGTAGAGGGTGT-3'	1462 (-1327 to +135)	94°C 20 sec, 58°C 30 sec, 68°C 1 min 30 sec 30 cycles
pGL3-Yy1-D	F:5'-CGGGGTACCTCGTCAGAGCAGACTTTATTGCG-3' R: 5'-CCCAAGCTTCGTGGCGATGTAGAGGGTGT-3'	979 (-844 to +135)	94°C 20 sec, 58°C 30 sec, 68°C 1 min 30 cycles
pGL3-Yy1-E	F:5'-CGGGGTACCTCGGGTTGAGGAGTTGGT-3' R: 5'-CCCAAGCTTCGTGGCGATGTAGAGGGTGT-3'	432 (-297 to +135)	94°C 20 sec, 58°C 30 sec, 68°C 30 sec 30 cycles

sample (2 µg) were reverse transcribed with Reverse Transcriptase System (Promega). The resulting cDNA samples were subjected to amplification using gene *Sohlh1* specific primers, Sohlh1F: 5' CGGGGTACCGCTTCCCTCCAACTT CGTGC 3', Sohlh1R: 5' CCGGAATC TCAGGGGAAAAAG TCAGGGGAAGATG 3' (KpnI site is underlined and EcoRI site is italicised) and Platinum® Pfx DNA Polymerase (Invitrogen). PCR production contained full-length CDS sequence of *sohlh1* was ligated into eukaryotic expression vector pcDNA3.0 to obtain recombinant plasmid pcDNA3.0-Sohlh1 which was identified by KpnI/EcoR I double digestion and nucleotide sequencing.

**Cell culture, transfection and luciferase assays:** Human Embryonic Kidney cells (HEK293) were grown in Dulbecco's modified Eagle's medium with 10% Fetal Bovine Serum (FBS). Cells (in 96 well plates) were cotransfected with *Sohlh1* expression plasmid DNA (100 ng) or pcDNA3.0 (100 ng) and each promoter construct (100 ng) using Lipofectamine 2000 transfection reagent according to the manufacturer's protocol. To normalize for transfection efficiency the cells were cotransfected with 20 ng of pRL-TK plasmid which encodes renilla luciferase. Firefly luciferase values were divided by renilla luciferase values to normalize for transfection efficiency.

About 44-48 h post-transfection, the cells were lysed with 20 µL 1 x passive lysis buffer, cultured for 15 min on a rocking platform at room temperature and then subsequently assayed for luciferase activity in cell lysate using the Dual Luciferase Reporter Assay System (Promega). Each 20 µL lysate was mixed with 80 µL of Luciferase Assay Reagent (LARII) in the 96 well multiwell plate which was then placed in the counter. Firefly luciferase activity was measured first followed by the addition of 80 µL stop/Glo® substrate to measure renilla luciferase. Statistical significance was determined using the paired t-test and the GraphPad Prism 5 Software package (GraphPad Software). The  $p < 0.05$  was deemed to indicate statistical significance.

**RESULTS AND DISCUSSION**

Kit is important for multiple aspects of oocyte and follicle development including the establishment of primordial germ cells within the ovary, primordial follicle activation, oocyte survival and growth (Hutt *et al.*, 2006). In previous study, the activation of the Kit promoter appears cell-specific in hematopoietic stem cells and mast cells (Maeda *et al.*, 2010). Because the composition of transcription factors is cell-specific, the Kit promoter in

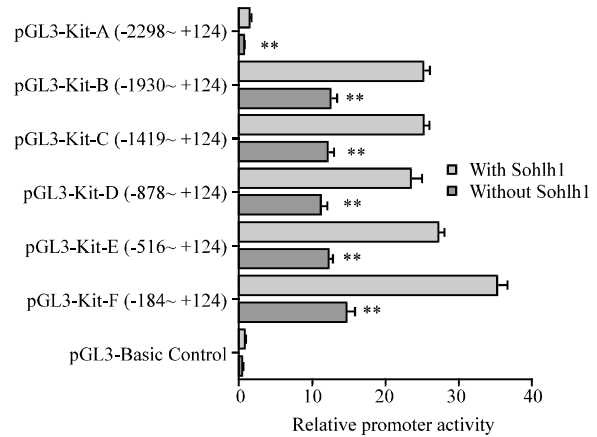


Fig. 1: The expression of *Sohlh1* causes activation of mouse Kit promoter. The Kit promoter-firefly luciferase vectors (pGL3-Kit-A-F) was co-transfected with either pcDNA 3.0-Sohlh1 or pcDNA 3.0. All transfections included the control vector pRL-TK that expresses renilla luciferase to correct for differences in transfection efficiency between samples. The activity of all promoter fragments stimulated by *Sohlh1* were significantly higher than those of *Sohlh1* absence. Data was analyzed with the paired t-test using GraphPad Prism Software, \* $p < 0.05$ , \*\* $p < 0.01$

oocytes possibly interacts with oocyte-specific transcription factors to appear different activity from hematopoietic stem cells and mast cells. We generated the reporter plasmid pGL3-Kit-A (-2298 to +124) and to screen cis-acting elements within the Kit promoter region, five deletion constructs carrying various lengths of 5'-flanking region were generated from -2298 bp. The programs of TFSEARCH (<http://mbs.cbrc.jp/research/db/TFSEARCH.html>) and WWW Promoter Scan (<http://www.bimas.nih.gov/molbio/proscan/>) were used to searching transcription factor binding Sites in Kit promoter. The program www promoter scan predicted promoter region in -1577 to -1327 in which a TATA-box (-1349) was found.

*Sohlh1* is one of the oocyte-specific gene expressed mainly in early stage of follicles play important and unique roles in primordial follicle formation, activation and survival (Jagarlamudi and Rajkovic, 2011). Figure 1 shows luciferase values of various lengths of Kit promoter in relative activity, cotransfected with or without *Sohlh1*. In the absence of *Sohlh1*, the relative luciferase activity of all Kit promoter fragments were over 20 fold higher than that of pGL3- Basic, except pGL3-Kit-A (2 fold). The promoter activity significantly increased by the deletion of -2298 to -1930, suggested that the -2298 to -1930 region contains powerful negative cis-regulatory element. In the -2298 to

-1930 region. There is one E2F binding motif. E2F transcription factors can interact with pocket proteins and possibly function as transcription repressors (Biyashev and Qin, 2011). The activity of pGL3-Kit-F (-184 to +124) activated by Sohlhl1 was highest >30 fold higher than that of pGL3-Basic. The program TFSEARCH revealed the presence of GATA and Sp1 binding motifs in the -184 to +124 region. GATA-1 and Sp1 are transcription factors with zinc finger domains for DNA binding. The interaction between the GATA-1 and Sp1 zinc finger domains is important for synergistic transcriptional effects (Imanishi *et al.*, 2010). In mast cells, the -232 to +22 region is essential for Kit promoter activity and GATA2 interacts with this promoter region via Sp1 and maintains expression of the *Kit* gene (Maeda *et al.*, 2010). In the presence of Sohlhl1, the activities of all promoter fragments were significantly higher than that of Sohlhl1 absence and the maximal stimulation was also in the -184 to +124 region. The result suggested Sohlhl1 could positively regulate the *Kit* promoter. Sohlhl1 encodes basic Helix-Loop-Helix (bHLH) transcriptional regulator which binding to a consensus sequence CANNTG called the E-box. There are six E-box in the -1419 to +124 region. In mouse ovary which E-box was bound by Sohlhl1 to activate *Kit* promoter requires further research.

Although, Yy1 has been reported to modulate a mounting list of genes such as c-myc, c-fos and p53, limited research has been carried out to investigate the mechanisms underlying how Yy1 is regulated even there is no study on the regulation of Yy1 promoter in oogenesis (Deng *et al.*, 2010). Figure 2 shows, regardless of the presence or absence of Sohlhl1, there was no difference between the activities of the same Yy1 promoter fragments except the pGL3-Yy1-E (-297 to +135). Although, significantly activated by Sohlhl1, the activity of pGL3-Yy1-E was drastically decreased which was <1/10 of those of pGL3-Yy1-B and pGL3-Yy1-C. The result suggested Sohlhl1 was without the function to affect the essential activation of Yy1 promoter. Figure 3 shown in the -297 to +135 region, the G/C content is 78%, markedly higher than that of -2202 to +135(57.6%). G/C-rich strands of DNA can adopt four-stranded structures known as G-quadruplexes (G4) motif (Hurley, 2001). In human YY1 promoter (-250 to 0) and 5'-UTR, the G/C content is 78.4 and 80%, respectively which is similar to that of mouse Yy1. Recent research revealed the presence of G4 structures in the promoter and the 5'-UTR of human YY1 gene and suggested an inhibitory role of the G4 motif in the YY1 promoter (Huang *et al.*, 2012). Researchers suspect that there should be G4 motifs near the transcription start site to inhibit the transcription

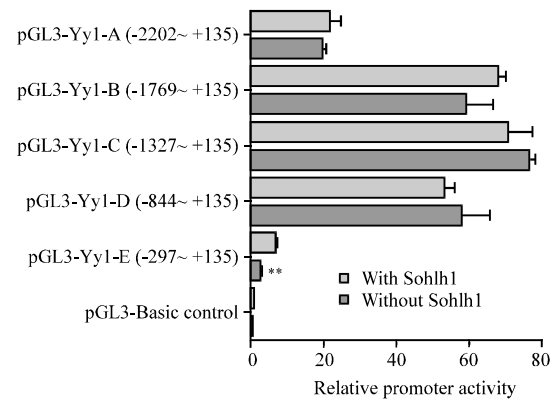


Fig. 2: Sohlhl1 has little capability to affect Yy1 transcription. Regardless of the presence or absence of Sohlhl1, there was no difference between the activities of the same Yy1 promoter fragments, except the pGL3-Yy1-E (-297 to +135). Although, significantly activated by Sohlhl1, the activity of pGL3-Yy1-E was drastically decreased which was much lower than those of the other promoters. Data was analyzed with the paired t-test using GraphPad Prism Software, \*p<0.05, \*\*p<0.01

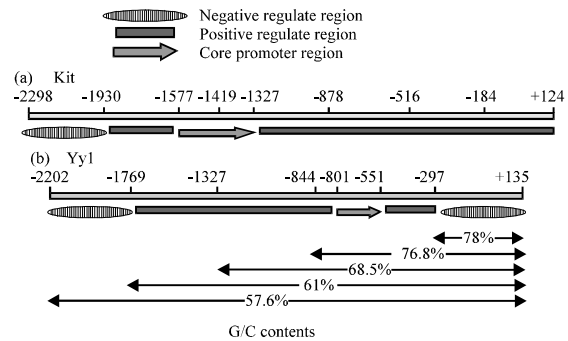


Fig. 3: Schematic map of mouse *Kit* and *Yy1* upstream regions; a) The program predicted *Kit* gene promoter region in -1577 to -1327 in which a TATA-box (-1349) was found. The promoter activity significantly increased by the deletion of -2298 to -1930, suggested that the -2298 to -1930 region contains powerful negative cis-regulatory element. The activity of pGL3-Kit-F (-184 to +124) activated by Sohlhl1 was highest; b) The G/C content is 78% in the -297 to +135 region of *Yy1* gene where possibly G4 motifs exist to inhibit the transcription activity, markedly higher than that of -2202 to +135(57.6%). The program predicted *Yy1* gene promoter region in -801 to -551. Deletion of the -1769 to -297 region resulted in the promoter activity drastically decreased

activity of mouse *Yy1*. The -1327 to +135 region (pGL3-*Yy1-C*) got the maximal activity and the program *www* promoter scan predicted promoter region in -801 to -551. Since, *Sohlhl* has little capability to affect *Yy1* transcription why *Sohlhl* was over-expressed in *Yy1* cKO ovaries? By using the program TESS (<http://www.cbil.upenn.edu/teess/>), researchers found two *Yy1* binding site(CCATNT) at -553 and -885 of *Sohlhl* promoter. *Yy1* can act as transcriptional activator and repressor whether *Yy1* negatively regulates *Sohlhl* in mouse ovary (Zhu *et al.*, 2011). However, this hypothesis requires further testing.

### CONCLUSION

In this study, *Kit* has been validated as *Sohlhl* responsive through promoter cloning and reporter gene analysis. Transient cotransfections of *Kit*-luciferase reporter vectors and *Sohlhl* expression vector provided evidence of *Sohlhl* transcriptional activation of the *Kit* gene promoter in the mouse ovary. Although, *Sohlhl* has little capability to affect *Yy1* transcription, we identified -1769 to -297 as the transcriptional activation region of *Yy1* promoter and revealed strong inhibitor in -297 to +135 region where maybe G4 motifs exist.

### ACKNOWLEDGEMENTS

This research was supported by the National Natural Science Foundation of China (30770818), Department of Education project of Liaoning province (2009s109).

### REFERENCES

- Biyashev, D. and G. Qin, 2011. E2F and microRNA regulation of angiogenesis. *Am. J. Cardiovasc. Dis.*, 1: 110-118.
- Brown, C., J. LaRocca, J. Pietruska, M. Ota and L. Anderson *et al.*, 2010. Subfertility caused by altered follicular development and oocyte growth in female mice lacking PKB  $\alpha$ /Akt1. *Biol. Reprod.*, 82: 246-256.
- Choi, Y., D. Yuan and A. Rajkovic, 2008b. Germ cell-specific transcriptional regulator *Sohlh2* is essential for early mouse folliculogenesis and oocyte-specific gene expression. *Biol. Reprod.*, 79: 1176-1182.
- Choi, Y., D.J. Ballow, Y. Xin and A. Rajkovic, 2008a. Lim homeobox gene, *lhx8*, is essential for mouse oocyte differentiation and survival. *Biol. Reprod.*, 79: 442-449.
- De Felici, M., 2000. Regulation of primordial germ cell development in the mouse. *Int. J. Dev. Biol.*, 44: 575-580.
- Deng, Z., P. Cao, M.M. Wan and G. Sui, 2010. Yin Yang 1: A multifaceted protein beyond a transcription factor. *Transcription.*, 1: 81-84.
- Driancourt, M.A., K. Reynaud, R. Cortvrindt and J. Smits, 2000. Roles of KIT and KIT LIGAND in ovarian function. *Rev. Reprod.*, 5: 143-152.
- Gilchrist, R.B., L.J. Ritter and D.T. Armstrong, 2004. Oocyte-somatic cell interactions during follicle development in mammals. *Anim. Reprod. Sci.*, 82-83: 431-446.
- Griffith, G.J., M.C. Trask, J. Hiller, M. Walentuk, J.B. Pawlak, K.D. Tremblay and J. Mager, 2011. Yin-yang1 is required in the mammalian oocyte for follicle expansion. *Biol. Reprod.*, 84: 654-663.
- Hinkins, M., J. Huntriss, D. Miller and H.M. Picton, 2005. Expression of polycomb-group genes in human ovarian follicles, oocytes and preimplantation embryos. *Reproduction*, 130: 883-888.
- Huang, W., P.J. Smaldino, Q. Zhang, L.D. Miller and P. Cao *et al.*, 2012. Yin Yang 1 contains G-quadruplex structures in its promoter and 5'-UTR and its expression is modulated by G4 resolvase 1. *Nucleic Acids Res.*, 40: 1033-1049.
- Hurley, L.H., 2001. Secondary DNA structures as molecular targets for cancer therapeutics. *Biochem. Soc. Trans.*, 29: 692-696.
- Hutt, K.J., E.A. McLaughlin and M.K. Holland, 2006. *Kit* ligand and *c-Kit* have diverse roles during mammalian oogenesis and folliculogenesis. *Mol. Hum. Reprod.*, 12: 61-69.
- Imanishi, M., C. Imamura, C. Higashi, W. Yan and S. Negi *et al.*, 2010. Zinc finger-zinc finger interaction between the transcription factors, GATA-1 and Sp1. *Biochem. Biophys. Res. Commun.*, 400: 625-630.
- Jagarlamudi, K. and A. Rajkovic, 2011. Oogenesis: Transcriptional regulators and mouse models. *Mol. Cell. Endocrinol.*
- Joshi, S., H. Davies, L.P. Sims, S.E. Levy and J. Dean, 2007. Ovarian gene expression in the absence of FIGLA, an oocyte-specific transcription factor. *BMC Dev. Biol.*, Vol. 7. 10.1186/1471-213X-7-67.
- Maeda, K., C. Nishiyama, H. Ogawa and K. Okumura, 2010. GATA2 and Sp1 positively regulate the *c-kit* promoter in mast cells. *J. Immunol.*, 185: 4252-4260.

- Pangas, S.A., Y. Choi, D.J. Ballow, Y. Zhao, H. Westphal, M.M. Matzuk and A. Rajkovic, 2006. Oogenesis requires germ cell-specific transcriptional regulators *Sohlh1* and *Lhx8*. *Proc. Natl. Acad. Sci.*, 103: 8090-8095.
- Rajkovic, A., S.A. Pangas, D. Ballow, N. Suzumori and M.M. Matzuk, 2004. *NOBOX* deficiency disrupts early folliculogenesis and oocyte-specific gene expression. *Science*, 305: 1157-1159.
- Xu, B., J. Hua, Y. Zhang, X. Jiang and H. Zhang *et al.*, 2011. Proliferating cell nuclear antigen regulates primordial follicle assembly by promoting apoptosis of oocytes in fetal and neonatal mouse ovaries. *PLoS One.*, Vol. 6, No. 1. 10.1371/journal.pone.0016046.
- Zhu, W., S.Y. Olson and H. Garban, 2011. Transcription regulator yin-yang 1: From silence to cancer. *Crit. Rev. Oncog.*, 16: 227-238.