Upregulated IncRNA Gm2044 inhibits male germ cell development by acting as miR-202 host gene

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ABSTRACT
Long non-coding RNAs (IncRNAs) have been found to participate in the regulation of human spermatogenic cell development. However, little is known about the abnormal expression of IncRNAs associated with spermatogenic failure and their molecular mechanisms. Using IncRNA microarray of testicular tissue for male infertility and bioinformatics methods, we identified the relatively conserved IncRNA Gm2044 which may play important roles in non-obstructive azoospermia. The UCSC Genome Browser showed that IncRNA Gm2044 is the miR-202 host gene. This study revealed that IncRNA Gm2044 and miR-202 were significantly increased in non-obstructive azoospermia of spermatogonial arrest. The mRNA and protein levels of Rbfox2, a known direct target gene of miR-202, were regulated by IncRNA Gm2044. Furthermore, the miR-202-Rbfox2 signalling pathway was shown to mediate the suppressive effects of IncRNA Gm2044 on the proliferation of human testicular embryonic carcinoma cells. Understanding of the molecular signalling pathways for IncRNA-regulated spermatogenesis will provide new clues into the pathogenesis and treatment of patients with male infertility.

Introduction
It has been reported that reproductive infertility occurs in 10–15% of couples in childbearing age, about half of which is caused by male factors including reproductive tract obstruction, inflammation, and sexual dysfunction (Matzuk and Lamb 2002; Pan et al. 2018). However, 60–75% of male infertility patients, known as idiopathic male infertility, are often accompanied by azoospermia or oligospermia, showing non-obstructive azoospermia (NOA). NOA is a complex disease caused by multiple factors with high genetic heterogeneity and phenotypic heterogeneity. Common genetic causes include chromosome abnormality, gene mutations, and epigenetic modifications (Shinjo et al. 2013; Vij et al. 2018). According to differences in spermatogenic disorders, NOA is mainly divided into maturation arrest, Sertoli cell only syndrome, and hypospermatogenesis. Spermatogenic maturation arrest, including early stage arrest (spermatogonial and spermatocyte stasis) and late stage arrest (sperm stasis), is one of the most complicated causes and most difficult to treat in male infertility. At present, the aetiology and mechanism of maturation arrest remain unknown in many spermatogenic arrest patients, which leads to great obstacles in the diagnosis and treatment of reproductive diseases (Miyamoto et al. 2012; Miyamoto et al. 2017). In-depth studies of spermatogenic maturation arrest regarding the related genetic factors, pathogenesis, and formation mechanism are needed.

LncRNAs are a class of endogenous non-coding RNAs with a length of more than 200 nucleotides and have been shown to participate in the regulation of human spermatogenic cell development (Kopp and Mendell 2018; Maeda et al. 2018). Research into the function and molecular mechanism of IncRNAs in male infertility is in the ascend. LncRNA AK015322 is highly expressed in spermatogonial stem cells and regulates the proliferation of spermatogonial stem cells by acting as a miR-19b-3p sponge (Hu et al. 2017). LncRNA H19 can regulate the proliferation and apoptosis of male germline stem cells via the IGF-1 signalling pathway (Lei et al. 2018). The IncRNA Mrhl inhibits the Wnt signalling pathway by interacting with the p68 protein and regulates Sox8 expression through binding to the Sox8 promoter in mouse spermatogonial cells, thus ensuring normal spermatogenesis (Arun et al. 2012; Kataruka et al. 2017). LncRNA NLC1-C is down-regulated in the testicular tissue of male infertility patients with spermatocyte maturation arrest and is involved in the regulation of spermatogenesis as competitive endogenous RNA of miR-302a and miR-383 (Lu et al. 2015). Male mice without IncRNA Tslrn1 show a significant decrease in spermatozoa production (Wichman et al. 2017).
miRNAs are a class of endogenous non-coding RNAs with a length of about 22 nucleotides and have been demonstrated to be involved in the regulation of germ cell development (Ambros 2004; Bartel 2004; Wu et al. 2012; Kotaja 2014; Wang and Xu 2015; Hilz et al. 2016). The testis-determining factor SOX9 binds to the pri-miR-202 promoter and then transcriptionally activates miR-202 expression in testis differentiation which suggest the crucial roles of SOX9-miR-202 signalling pathway during testis development (Wainwright et al. 2013). Furthermore, miR-202 mediates the GDNF and RA (retinoic acid) regulatory network and affects the self-renewal and differentiation of spermatogonial stem cells by directly targeting Rbfox2 (RNA binding fox-1 homolog 2) (Chen et al. 2017). In zebrafish, miR-202 is mainly expressed in oocytes during follicular development and exhibits a typical primordial germ cell-specific expression marker throughout embryogenesis (Zhang et al. 2017). Deletion of miR-202 in female medaka led to no egg production or dramatically decreased the number of eggs that could not be fertilised, ultimately resulting in no successful pregnancy (Gay et al. 2018). In addition, upregulated miR-202 enhances the progression of endometriosis by directly targeting SOX6 and its downstream pathways including p21, cyclin D1, and pRb (Zhang et al. 2015). miR-202 has been confirmed to function as a tumour suppressor in the progression of endometrial adenocarcinoma by targeting the oncogene FOXR2 (Deng et al. 2017). The IncRNA NORAD promotes colorectal cancer cell proliferation, migration, and invasion and is associated with a poor prognosis by acting as an endogenous competitor of RNA of miR-202 (Zhang et al. 2018b). Taken together, miR-202 play critical roles in the activity, health, and disease of human beings.

In this study, the testicular tissues of male infertility patients were used to reveal abnormal expression status using IncRNA microarray. The results suggest that IncRNA may be involved in the process of spermatogenic differentiation and meiosis. We identified the abnormal expression of the conservative IncRNA Gm2044, miR-202, and Rbfox2 (a known direct target gene of miR-202) in non-obstructive azoospermia in spermatogonial arrest, and found that the miR-202-Rbfox2 signalling molecular pathway mediates the suppressive effects of IncRNA Gm2044 on the proliferation of the human testicular embryonic carcinoma cell line NCCIT.

Materials and methods

Cell culture and transfection

NCCIT cells were grown in RPMI-1640 medium (Life Technologies, Carlsbad, CA, USA) supplemented with 10% (v/v) foetal bovine serum (Life Technologies) and 1% penicillin–streptomycin (100 U/ml penicillin and 100 μg/ml streptomycin) (Life Technologies) at 37°C in 5% carbon dioxide incubator. Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) was selected to transfect the NCCIT cells following the manufacturer’s instructions.

RNA extract and RT-qPCR

Total RNA was extracted using Trizol (Invitrogen) from testicular tissues and NCCIT cells. The relative expression levels of IncRNA Gm2044, Rbfox2 mRNA, and miR-202 were analysed by RT-qPCR according to our previous studies (Hu and Liang 2017; Hu et al. 2018).

Western blotting

The protein of testicular tissues and NCCIT cells was isolated using radio immunoprecipitation assay lysis buffer (Millipore, Bedford, MA, USA) with a 1% protease inhibitor cocktail (Roche, Indianapolis, IN, USA), separated on SDS-PAGE, transferred onto nitrocellulose membrane filters (Amersham Biosciences, Freiburg, Germany), and then immunoblotted with antibody. Anti-RBFOX2 and anti-β-actin antibodies were obtained from Thermo Fisher Scientific (Rockford, IL, USA).

Cell proliferation analysis

CCK-8 reagent (Dojindo Laboratories, Kumamoto, Japan) was used to determine the proliferation of transfected NCCIT cells according to the manufacturer’s protocol. The absorbance at 450 nm in transfected NCCIT cells was detected using a microplate reader.

Statistics

Each experiment was performed at least three times. The data were analysed using Student’s t-test or ANOVA, and are presented as mean ± SEM. Differences were considered to be statistically significant when P < 0.05.

Results

IncRNA Gm2044 and miR-202 are highly expressed in non-obstructive azoospermia with spermatogonial arrest

Using an IncRNA microarray on testicular tissue obtained from male infertility patients and bioinformatics methods, we identified the relatively conserved IncRNA Gm2044, which may play important roles in non-obstructive azoospermia (NOA). The relative expression of
IncrRNA Gm2044 in obstructive azoospermia (OA), NOA with spermatogonial arrest (NOA-Spg), NOA with spermatocyte arrest (NOA-Spc), and hypospermatogenesis (Hypo) was measured by RT-qPCR. The results show that IncrRNA Gm2044 was significantly increased in NOA-Spg (Figure 1(A)). Additionally, IncrRNA Gm2044 was weakly expressed in spermatogonia and highly expressed in healthy spermatocytes (Hu et al. 2018). The abnormal expression of IncrRNA Gm2044 in spermatogonia cells in NOA-spg male infertility testicular tissue may play a critical role in blocking spermatogonia cell development.

The UCSC Genome Browser showed that IncrRNA Gm2044 is the miR-202 host gene. RT-qPCR demonstrated that miR-202 was also highly expressed in NOA-spg male infertility testicular tissue (Figure 1(B)). Previous research found that miR-202 can directly target Rbfox2 and regulate spermatogenesis (Chen et al. 2017). This study revealed that the expression level of RBFOX2 protein was significantly lower in NOA-spg male infertility testicular tissue by western blotting analysis (Figure 1(C)).

**LncrRNA Gm2044 inhibits Rbfox2 expression**

To determine whether IncrRNA Gm2044 regulates Rbfox2 expression through miR-202, overexpression of Gm2044 was carried out in human testicular embryonic carcinoma cells (NCCIT) by transfection with pcDNA3.1 (+)-Gm2044 plasmid. The result demonstrated that overexpression of Gm2044 led to a high level of miR-202.

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**Figure 1.** The relative expression of IncrRNA Gm2044 and miR-202. A and B, LncrRNA Gm2044 (A) and miR-202 (B) were significantly increased in NOA-Spg. Total RNA was isolated from OA, NOA-Spg, NOA-Spc, and Hypo, and then subjected to RT-qPCR analysis for IncrRNA Gm2044 (A) and miR-202 (B). C, RBFOX2 protein was significantly decreased in NOA-Spg. Protein was extracted from OA, NOA-Spg, NOA-Spc, and Hypo, and then subjected to western blotting analysis for RBFOX2 and β-actin. The top panel shows the band of western blotting and the bottom panel gives the statistical summary of the above band. In A and B, β-actin mRNA and U6 snRNA were used as the reference genes, respectively. OA, obstructive azoospermia; NOA-Spg, non-obstructive azoospermia of spermatogonial arrest; NOA-Spc, non-obstructive azoospermia of spermatocyte arrest; Hypo, hypospermatogenesis; **, $P \leq 0.01$; ***, $P \leq 0.001$. 
which may due to the transformation of Gm2044 (Figure 2(A)). Overexpression of IncRNA Gm2044 significantly decreased the Rbfox2 mRNA and protein level (Figure 2 (A and B)). However, knockdown of miR-202 rescued the suppressive effects of IncRNA Gm2044 on Rbfox2 mRNA and protein expression (Figure 2(A and B)). These results suggest that miR-202 can mediate the effect of upregulated IncRNA Gm2044 in NOA-Spg on regulating Rbfox2 expression.

The miR-202-Rbfox2 signalling mediates the inhibitory effects of IncRNA Gm2044 on NCCIT cell proliferation

Human testicular embryonic carcinoma cells (NCCIT, a pluripotent extragonadal germ cell tumour cell line) was used to study the effect of IncRNA Gm2044 in vitro. Overexpression of IncRNA Gm2044 in NCCIT cells significantly inhibited cell proliferation (Figure 3(A and B)). Knockdown of miR-202 (Figure 3(A)) or overexpression of Rbfox2 (Figure 3(B)) attenuated the inhibitory effects of IncRNA Gm2044 on NCCIT proliferation. Taken together, the miR-202-Rbfox2 molecular signalling pathway appears to mediate the inhibitory effects of IncRNA Gm2044 on the proliferation of human testicular embryonic carcinoma cells.

Discussion

In recent years, we have focused on studies of IncRNAs in spermatogenesis (Liang et al. 2014; Hu et al. 2017; Hu et al. 2018). The expression of IncRNAs and mRNAs in male germ cells at different critical stages was analysed by microarray. The results show that IncRNAs and mRNAs show coordinated expression (Liang et al. 2014). High levels of the IncRNA AK015322 enhance spermatogonial stem cell (C18-4) proliferation by regulating the miR-19b-3p-ETV5 signalling pathway (Hu et al. 2017). In addition, we also found that IncRNA Gm2044 was enriched in spermatocytes and inhibited translation of the adjacent reproductive gene Utf1 by interacting with Utf1 mRNA (Hu et al. 2018). In this study, we

Figure 2. LncRNA Gm2044 suppresses the Rbfox2 expression. A, The level of Rbfox2 mRNA was inhibited by overexpression of lncRNA Gm2044 and rescued by knockdown of miR-202. NCCIT cells transfected with pcDNA3.1(+)/pcDNA3.1(+)-Gm2044/inhibitor NC/miR-202 inhibitor were used to isolate RNA, and then subjected to RT-qPCR detection for lncRNA Gm2044 and miR-202. B, The level of RBFOX2 protein was inhibited by overexpression of lncRNA Gm2044 and rescued by knockdown of miR-202. NCCIT cells transfected with pcDNA3.1(+)/pcDNA3.1(+)-Gm2044/inhibitor NC/miR-202 inhibitor were used to extract protein, and then subjected to western blotting for RBFOX2 and β-actin. The top panel shows the band of western blotting and the bottom panel gives the statistical summary of the above band. NC, negative control; **, P ≤ 0.01; ***, P ≤ 0.001.
mainly explored the roles of IncRNA in male infertility. Upregulated IncRNA Gm2044 was found to be a potential cause of impaired spermatogonial development.

The transition of spermatogonia to spermatocytes is a regulatory process that involves germ cell differentiation from spermatogonial stem cells (As, a single spermatogonia) (Raverdeau et al. 2012). The spermatogonial stem cell divides into two paired A (Ap) spermatogonia and then to 4–32 aligned (Aal) spermatogonia (de Rooij and Russell 2000; Nakagawa et al. 2010). Subsequently, Aal spermatogonia in turn give rise to A1, A2, A3, A4, In, and B spermatogonia, which then generate premeiotic spermatocytes. LncRNA033862 regulates the self-renewal and survival of spermatogonial stem cells by interaction with Gfra1 chromatin (Li et al. 2016). LncRNA Mrhl can mediate the function of Wnt signalling pathway in mouse spermatogonia (Akhade et al. 2016). LncRNA HSVIII plays crucial roles in spermatocyte meiosis by affecting Prss42 and Tessp2 expression (Yoneda et al. 2016). However, little is known about the regulation of IncRNAs in the transition of spermatogonia to spermatocytes. This study revealed that IncRNA Gm2044 was significantly increased in NOA-Spg and led to abnormal expression of the miR-202-Rbfox2 molecular pathway, indicating that IncRNA Gm2044 and its downstream signalling have the ability to regulate male infertility.

Studies on the mechanism of the mutual regulation for IncRNAs and miRNAs have drawn considerable attention in cellular development (Janakiraman et al. 2018; Xiao et al. 2018). IncRNAs/miRNAs interact with RNA binding proteins to mediate the TGF-β molecular signalling pathway during post-transcriptional genetic regulation (Janakiraman et al. 2018). Because the abnormal expression and dysregulation of IncRNAs and miRNAs occurs in cancer, IncRNAs-miRNAs have been identified as biomarkers in certain cancers (Zhang et al. 2018a). LncRNA MEG3 is down-regulated in gastric cancer and plays critical roles in gastric cancer by suppressing miR-21 expression (Dan et al. 2018). Hypermethylation of CpG islands on the promoter lead to the loss of miR-31 and its host gene IncRNA LOC554202 in triple-negative breast cancer, which then promotes breast cancer growth and metastasis (Augoff et al. 2012). LncRNA MIR100HG, an mRNA-host gene IncRNA, interacts with HuR/ELAVL1 to modify RNA-binding protein ability in human cells (Sun et al. 2018). LncRNA Gm2044 is the miR-202 host gene, and we demonstrated that IncRNA Gm2044 and miR-202 are significantly upregulated in NOA-Spg male infertility testicular tissue. miR-202 can mediate the function of upregulated IncRNA Gm2044 in NOA-Spg by regulating Rbfox2 expression and modulating NCCIT cell proliferation.

In summary, IncRNA Gm2044 is significantly elevated in NOA-Spg and suppresses Rbfox2 expression by acting as miR-202 host gene. Furthermore, the miR-202-Rbfox2 molecular signalling pathway mediates the inhibitory effects of IncRNA Gm2044 on the proliferation of the human testicular embryonic carcinoma cell NCCIT. Understanding the signalling pathway for IncRNAs in male reproduction will provide new clues to the pathogenesis and treatment of male infertility.

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Compliance with ethical standards

This study received ethical approval from the institutional review board of Bengbu Medical College.

Author contributions

Meng Liang and Yaping Liao conceived of the study; Meng Liang performed the research and wrote the manuscript; Ke Hu analysed the results; Chaofan He and Jinzhao Zhou reviewed the manuscript.
**Disclosure statement**
No potential conflict of interest was reported by the authors.

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**References**
Akhade VS, Dighe SN, Kataruka S, Rao MR. 2016. Mechanism of Wnt signaling induced down regulation of mrhl long non-coding RNA in mouse spermatogonial cells. Nucleic Acids Res. 44:350–355.

Ambros V. 2004 Sep 16. The functions of animal microRNAs. Nature. 431:350–355. Epub 2004/09/17.

Arun G, Akhade VS, Donakonda S, Rao MR. 2012. mrhl RNA, a long non-coding RNA, negatively regulates Wnt signaling through its protein partner Ddx5/p68 in mouse spermatogonial cells. Mol Cell Biol. 32:3140–3152.

Augoff K, McCue B, Plow EF, Sossey-Alaoui K. 2012. miR-31 and its host gene IncRNA LOC554202 are regulated by promoter hypermethylation in triple-negative breast cancer. Mol Cancer. 11:5.

Bartel DP. 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 116:281–297.

Chen J, Cai T, Zheng C, Lin X, Wang G, Liao S, Wang X, Gan H, Zhang D, Hu X, et al. 2017. MicroRNA-202 maintains spermatogonial stem cells by inhibiting cell cycle regulators and RNA binding proteins. Nucleic Acids Res. 45:4142–4157.

Dan J, Wang J, Wang Y, Zhu M, Yang X, Peng Z, Jiang H, Chen L. 2018. LncRNA-MEG3 inhibits proliferation and metastasis by regulating miRNA-21 in gastric cancer. Biomed Pharmacother. 99:931–938.

Deng X, Hou C, Liang Z, Wang H, Zhu L, Xu H. 2017. miR-202 suppresses cell proliferation by targeting FOXR2 in endometrial adenocarcinoma. Dis Markers. 2017:2827435.

de Rooij DG, Russell LD. 2000. All you wanted to know about spermatogonia but were afraid to ask. J Androl. 21:776–798.

Gay S, Bugeon J, Bouchareb A, Henry L, Delahaye C, Legeai F, Montfort J, Le Cam A, Siegel A, Bobe J, et al. 2018. MIr-202 controls female fecundity by regulating medaka oogenesis. PLoS Genet. 14:e1007593.

Hilz S, Modzelewski AJ, Cohen PE, Grimson A. 2016. The roles of microRNAs and siRNAs in mammalian spermatogenesis. Development. 143:3061–3073.

Hu K, Li L, Liao Y, Liang M. 2018. LncRNA Gm2044 highly expresses in spermatocyte and inhibits Utf1 translation by interacting with Utf1 mRNA. Genes Genomics. 40:781–787.

Hu K, Liang M. 2017. Upregulated microRNA-224 promotes ovarian cancer cell proliferation by targeting KLLN. In Vitro Cell Dev Biol Anim. 53:149–156.

Hu K, Zhang J, Liang M. 2017. LncRNA AK015322 promotes proliferation of spermatogonial stem cell C18-4 by acting as a decoy for microRNA-19b-3p. In Vitro Cell Dev Biol Anim. 53:277–284.

Janakiraman H, House RP, Gangaraju VK, Diehl JA, Howe PH, Palanisamy V. 2018. The long (IncRNA) and short (miRNA) of it: TGFbeta-mediated control of RNA-binding proteins and noncoding RNAs. Mol Cancer Res: MCR. 16:567–579.

Kataruka S, Akhade VS, Kayyar B, Rao MRS. 2017. Mrhl long non-coding RNA mediates meiotic commitment of mouse spermatogonial cells by regulating Sox8 expression. Mol Cell Biol. 15:37.

Kopp F, Mendell JT. 2018. Functional classification and experimental dissection of long noncoding RNAs. Cell. 172:393–407.

Kotaja N. 2014. MicroRNAs and spermatogenesis. Fertil Steril. 101:1552–1562.

Lei Q, Pan Q, Li N, Zhou Z, Zhang J, He X, Peng S, Li G, Sidhu K, Chen S, et al. 2018. H19 regulates the proliferation of bovine male germine stem cells via IGF-1 signaling pathway. J Cell Physiol. 234(1):915–926. Aug 1.

Li L, Wang M, Wu X, Geng L, Xue Y, Wei X, Jia Y. 2016. A long non-coding RNA interacts with Gfra1 and maintains survival of mouse spermatogonial stem cells. Cell Death Dis. 7: e2140.

Liang M, Li W, Tian H, Hu T, Wang L, Lin Y, Li Y, Huang H, Sun F. 2014. Sequential expression of long noncoding RNA as mRNA gene expression in specific stages of mouse spermatogenesis. Sci Rep. 4:5966.

Lu M, Tian H, Cao YX, He X, Chen L, Song X, Ping P, Huang H, Sun F. 2015. Downregulation of miR-320a/383-sponge-like long non-coding RNA NCLC1–C (narcolepsy candidate-region 1 genes) is associated with male infertility and promotes testicular embryonal carcinoma cell proliferation. Cell Death Dis. 6:e1960.

Maeda RK, Sitnik JL, Frei Y, Prince E, Gligorov D, Wolfner MF, Karch F. 2018. The IncRNA male-specific abdominal plays a critical role in Drosophila accessory gland development and male fertility. PLoS Genet. 14:e1007519.

Matzuk MM, Lamb DJ. 2002. Genetic dissection of mammalian fertility pathways. Nat Cell Biol. 4:s41–s49.

Myamoto T, Minase G, Shin T, Ueda H, Okada H, Sengoku K. 2017. Human male infertility and its genetic causes. Reprod Med Biol. 16:81–88.

Myamoto T, Tsujimura A, Miyagawa Y, Koh E, Namiki M, Sengoku K. 2012. Male infertility and its causes in human. Adv Urol. 2012:384520.

Nakagawa T, Sharma M, Nabeeshima Y, Braun RE, Yoshida S. 2010. Functional hierarchy and reversibility within the murine spermatogenic stem cell compartment. Science. 328:62–67.

Pan MM, Hockenberry MS, Kirby EW, Lipshultz LI. 2018. Male infertility diagnosis and treatment in the era of in vitro fertilization and intracytoplasmic sperm injection. Med Clin North Am. 102:337–347.

Raverdeau M, Gely-Pernot A, Gosset B, Denefeld C, Belloir G, Davidson I, Chambon P, Mark M, Ghyselinck NB. 2012. Retinoic acid induces Sertoli cell paracrine signals for spermatogenesis and male fertility. PLoS Genet. 101:1552–1562.

Shinjo E, Shiraishi K, Matsuyama H. 2013. The effect of human chorionic gonadotropin-based hormonal therapy on intra-testicular testosterone levels and spermatogonial DNA synthesis in men with non-obstructive azoospermia. Andrology. 1:929–935.
Sun Q, Tripathi V, Yoon JH, Singh DK, Hao Q, Min KW, Davila S, Zealy RW, Li XL, Polycarpou-Schwarz M, et al. 2018. MIR100 host gene-encoded lncRNAs regulate cell cycle by modulating the interaction between HuR and its target mRNAs. Nucleic Acids Res. 46:10405–10416.

Vij SC, Sabanegh Jr. E, Agarwal A. 2018. Biological therapy for non-obstructive azoospermia. Expert Opin Biol Ther. 18:19–23.

Wainwright EN, Jorgensen JS, Kim Y, Truong V, Bagheri-Fam S, Davidson T, Svingen T, Fernandez-Valverde SL, McClelland KS, Taft RJ, et al. 2013. SOX9 regulates microRNA miR-202-5p/3p expression during mouse testis differentiation. Biol Reprod. 89:34.

Wang L, Xu C. 2015. Role of microRNAs in mammalian spermatogenesis and testicular germ cell tumors. Reproduction. 149:R127–R137.

Wichman L, Somasundaram S, Breindel C, Valerio DM, McCarrey JR, Hodges CA, Khalil AM. 2017. Dynamic expression of long noncoding RNAs reveals their potential roles in spermatogenesis and fertility. Biol Reprod. 97:313–323.

Wu W, Hu Z, Qin Y, Dong J, Dai J, Lu C, Zhang W, Shen H, Xia Y, Wang X. 2012. Seminal plasma microRNAs: potential biomarkers for spermatogenesis status. Mol Hum Reprod. 18:489–497.

Xiao B, Zhang W, Chen L, Hang J, Wang L, Zhang R, Liao Y, Chen J, Ma Q, Sun Z, et al. 2018. Analysis of the miRNA-mRNA-lncRNA network in human estrogen receptor-positive and estrogen receptor-negative breast cancer based on TCGA data. Gene. 658:28–35.

Yoneda R, Satoh Y, Yoshida I, Kawamura S, Kotani T, Kimura AP. 2016. A genomic region transcribed into a long noncoding RNA interacts with the Prss42/Tessp-2 promoter in spermatocytes during mouse spermatogenesis, and its flanking sequences can function as enhancers. Mol Reprod Dev. 83:541–557.

Zhang J, Li XY, Hu P, Ding YS. 2018b. LncRNA NORAD contributes to colorectal cancer progression by inhibition of miR-202-5p. Oncol Res. 22.

Zhang D, Li Y, Tian J, Zhang H, Wang S. 2015. MiR-202 promotes endometriosis by regulating SOX6 expression. Int J Clin Exp Med. 8:17757–17764.

Zhang J, Liu W, Jin Y, Jia P, Jia K, Yi M. 2017. MiR-202-5p is a novel germ plasm-specific microRNA in zebrafish. Sci Rep. 7:7055.

Zhang G, Pian C, Chen Z, Zhang J, Xu M, Zhang L, Chen Y. 2018a. Identification of cancer-related miRNA-lncRNA biomarkers using a basic miRNA-lncRNA network. PloS One. 13: e0196681.