**Tet1 Deficiency Leads to Premature Ovarian Failure**

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Tet enzymes participate in DNA demethylation and play critical roles in stem cell pluripotency and differentiation. DNA methylation alters with age. We find that Tet1 deficiency reduces fertility and leads to accelerated reproductive failure with age. Noticeably, Tet1-deficient mice at young age exhibit dramatically reduced follicle reserve and the follicle reserve further decreases with age, phenomenon consistent with premature ovarian failure (POF) syndrome. Consequently, Tet1-deficient mice become infertile by reproductive middle age, while age matched wild-type mice still robustly reproduce. Moreover, by single cell transcriptome analysis of oocytes, Tet1 deficiency elevates organelle fission, associated with defects in ubiquitination and declined autophagy, and also upregulates signaling pathways for Alzheimer’s diseases, but down-regulates X-chromosome linked genes, such as Fmr1, which is known to be implicated in POF. Additionally, Line1 is aberrantly upregulated and endogenous retroviruses also are altered in Tet1-deficient oocytes. These molecular changes are consistent with oocyte senescence and follicle atresia and depletion found in premature ovarian failure or insufficiency. Our data suggest that Tet1 enzyme plays roles in maintaining oocyte quality as well as oocyte number and follicle reserve and its deficiency can lead to POF.

Keywords: Tet1, aging, epigenetics, oocyte, premature ovarian failure

**INTRODUCTION**

Ten-eleven translocation (Tet) methylcytosine dioxygenases play a major role in shaping DNA methylation patterns through demethylation (Wu and Zhang, 2017). Tet enzymes have been demonstrated to play important roles in ESC pluripotency and differentiation (Ito et al., 2010; Koh et al., 2011). However, Tet1 or Tet2 -null mice are viable and overtly normal double Tet1/Tet2-deficient mice are also obtained (Dawlaty et al., 2011, 2013; Ko et al., 2011), but, some Tet1-deficient mice display a smaller body size at birth, which might reflect a developmental delay. Moreover, Tet1-mediated 5 hmC signals play important role in DNA demethylation during primordial germ cell (PGC) development and meiosis (Yamaguchi et al., 2012, 2013a; Hill et al., 2018). Tet1 deficiency results in meiotic defects of PGCs, including impaired homologous pairing and recombination in meiotic germ cells (meiocytes) during fetal development, probably due to insufficient demethylation and failed activation of meiotic genes (Yamaguchi et al., 2013a), such that Tet1-deficient mice at young age already exhibit reduced number of oocytes in the ovary and subfertility (Yamaguchi et al., 2012, 2013a). It also will be interesting to investigate whether Tet1 deficiency impacts reproductive aging of adult mice.
From the onset of reproductive maturity, organismal aging is characterized by declined fecundity, increased tissue dysfunction, and susceptibility to disease or mortality (Rando and Chang, 2012). The aging process is associated with altered epigenetic regulation, including DNA methylation, histone modification and chromatin remodeling (Zhang et al., 2020). DNA methylation involves in many aspects of cellular and molecular changes in aging (Lopez-Otin et al., 2013). Moreover, change to DNA methylation in aging has been used to predict the chronological age of human somatic tissues and individuals (Hannum et al., 2013; Horvath, 2013). Predictions have been made from heterogeneous samples such as lung, liver, and brain tissues as well as whole blood and peripheral blood mononuclear cells, and isolated CD4 + T cells, monocytes, and B cells (Horvath, 2013). Epigenetic clock sites have been defined also in multiple tissues in mouse (Stubbs et al., 2017) and age-related methylation changes between mouse and human were partially conserved (Maegawa et al., 2010). Deeper understanding of epigenetic alterations in aging and the molecular basis of the DNA methylation clock is of particular interest as epigenetic modifications can be reversed, allowing manipulation to potentially reverse aging and thus therapeutic potential (Rando and Chang, 2012; Cole et al., 2017; Chen and Kerr, 2019; Zhang et al., 2020). Nevertheless, it remains elusive whether Tet deficiency can impact aging in the adult.

Ovarian aging is mainly characterized by a sharp decrease in the number of oocyte and follicles and a decrease in oocyte quality (van Rooij et al., 2005; Broekmans et al., 2007, 2009; Tatone et al., 2008). The number of oocytes and follicles stored known as follicle reserve at birth is fixed, which cannot be replenished by germ cells in postnatal ovaries, but with age, periodic ovulation, follicle atresia and apoptosis are the main causes of a sharp decline in the number of follicles (Faddy, 2000; Bristol-Gould et al., 2006; Broekmans et al., 2007; Djahanbakhch et al., 2007; Notarianni, 2011; Zhang et al., 2012). Follicle depletion, in turn, causes lower levels of estrogen secretion in the body, eventually leading to menopause (Lawson et al., 2003).

Recently, we report that Tet2 deficiency accelerates reproductive aging in the adult female mice (Wang et al., 2020). Here we compared the fertility of mice deficient for Tet1 from young to reproductive old age. We report roles of Tet1 in maintaining oocyte quality in addition to oocyte number for preserving fertility with age.

**MATERIALS AND METHODS**

**Tet1 Knockout Mice**

Tet1 knockout (Tet1<sup>-/-</sup>) mice were generated from 129 × C57BL/6J mixed genetic background (Zhang et al., 2013). Most of wild-type (WT) and Tet1 knockout mice were generated from Tet1 heterozygotes mating. For fertility analysis, Tet1<sup>-/-</sup> females were crossed with WT male at young age and vaginal plugs were checked daily. All mice in the study were maintained in a mixed 129 × C57BL/6J background. All mouse experiments were carried out in accordance with the guidelines and regulations and approved by the Institutional Animal Care and Use Committee of Nankai University.

**Genotyping**

Postnatal mice at 2 weeks old were genotyped using DNA extracted from their ears. PCR was carried out at 94°C for 2 min, followed by 35 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min. DNA fragments were visualized by agarose gel electrophoresis. The genotyping primers were listed as follows.

| Tet1-C  | CAGTACGATTGGCGCTGCTATACAT |
| Tet1-R  | TTCCCTAGGAGTTACTCAGAACGG |
| Tet1-F  | CATCTAAAATACCCCAACCAACAA |

**H&E of Ovary and Follicle Count**

Ovaries were collected from different group mice (n > 10 mice for young and middle-age group and n = 7–8 mice for old group) and fixed by immersion in 4% paraformaldehyde (PFA) overnight at 4°C, and tissues were embedded with paraffin wax, based on previous methods (Liu et al., 2013). The follicles were categorized into primordial and primary, secondary and antral, accordingly (Myers et al., 2004). Follicles were classified as primordial and primary if they contained an oocyte surrounded by a single layer of squamous, or, cuboidal granulosa cells. Secondary follicles were identified as having more than one layer of granulosa cells with no visible antrum. Antral follicles possessed one or two small areas of follicular fluid (antrum) or a single large antral space.

**Oocyte Collection**

For collection of oocytes for RNA-seq, female mice from the different groups were superovulated by intraperitoneal injection of 5 IU pregnant mare’s serum gonadotrophin (PMSG), followed 46–48 h later by 5 IU human chorionic gonadotrophin (hCG), to obtain MII oocytes. Granulosa cells attached to oocytes were removed in M2, or HKSOM supplemented with 0.03% hyaluronidase by gently pipetting.

**Quantitative Real-Time PCR**

RNA was isolated from ovaries using RNeasy mini kit (Qiagen), and subject to cDNA synthesis using Moloney Murine Leukemia Virus Reverse Transcriptase (Invitrogen). PCR reactions were set up in duplicates using the FastStart Universal SYBR Green Master (493914001, Roche) and run on the Mastercycler<sup>®</sup> RealPlex2 real time PCR detection system (Eppendorf). The final PCR reaction volume in 20 µl contained 10 µl SYBR Green PCR Master Mix, 1 µl cDNA template, 2 µl primer mixture and 7 µl water. Thermal cycling was carried out with a 10 min denaturation step at 95°C, followed by two-step cycles, 15 s at 95°C and 1 min at 60°C. Each sample was analyzed using GAPDH as the internal control. Primers were designed as follows.

| Tet1-Foward  | CCTCACAGGCAACCAGGTACAA |
| Tet1-Reverse | ATTGGGCAGCTTTATCTGCT |
| Gapdh-Foward | TCAACAGCAAATCCCACTTCTCA |
| Gapdh-Reverse | ACCACCCGCTGCTGCTGCTGCT |

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Immunofluorescence of Spreads, Sections, and Oocytes

For paraffin sections, after deparaffinizing, rehydrating and washing in PBS (pH 7.2–7.4), the sections were subjected to high pressure antigen recovery sequentially in citrate buffer (pH 6.0) for 3 min, incubated with blocking solution (5% goat serum and 0.1% BSA in PBS) for 2 h at room temperature, and then incubated with the diluted primary antibodies [anti-5 hmC (39769, Active Motif), anti-Tet1 (MABE1144, Millipore), anti-Oct4 (SC-5279, Santa Cruz)] overnight at 4°C. After washing with PBS, sections were incubated with appropriate secondary antibodies (Alexa Fluor® FITC, 488, or 594). The sections were then stained with 1 µg/ml DAPI for 10 min to reveal nuclei, washed with PBS, and mounted in Vectashield (H-1000, Vector Laboratories, Burlingame, CA, United States). Relative integrated fluorescence intensity for 5 hmC was estimated by Image J software. The threshold was defined using non-specific background fluorescence.

Single-Cell Isolation and Lysis

MII oocytes were collected from young WT (n = 19), Tet1−/− (n = 15) or from old WT (n = 19), Tet1−/− (n = 14) mice in two biologically repeated experiments. Single MII oocyte was resuspended in PBS with 0.1% BSA (A3311-10g, Sigma), picked up in 1 µL 0.1% BSA using a micropipette with an epT.I.P.S. pipette tip (0030000838, Eppendorf) under a dissecting microscope, and transferred to the bottom of a 200-µL PCR tube (8-strip, nuclease-free, thin-walled PCR tubes with caps, PCR-0208-C, Axygen) containing oligo (dT) primer, Triton X-100, and Recombinant RNase Inhibitor (RRI, 2313A, Takara). Samples were frozen in liquid nitrogen and stored or used immediately.

Reverse Transcription

Frozen or fresh samples were melted on ice and 1 µL deoxyribonucleoside triphosphate (R0191, Thermo Scientific) was added into the tubes, vortexed gently, and incubated at 37°C for 3 min. The cDNAs were then synthesized using SuperScript™ II Reverse Transcriptase (18064071, Thermo Scientific) by slightly modified Smart-seq2 methods (Picelli et al., 2014), followed by 15 cycles of PCR using KAPA HotStart ReadyMix (KK2602, KAPA Biosystems) and then purified using Agencount AMPure XP beads (A63881, Beckman).

Library Construction and Sequencing

RNA-seq libraries were prepared using Smart-seq2 methods as previously described (Picelli et al., 2014). Briefly, the libraries were prepared by using TruePrep DNA Library Prep Kit V2 for Illumina® (TD503-02, Vazyme Biotech co., Ltd.) according to the manual instruction. Samples were barcoded during library preparation and multiplex sequenced, with a 150-bp pair-end sequencing strategy on a HiSeq 10x (Illumina).

Single-Cell RNA-seq Data Analysis

Raw reads were processed using trim-galore and clean reads were mapped to mm 10 from UCSC genome1 by

1http://genome.ucsc.edu/

Analysis of Transposable Elements

Reads were mapped to mm 10 from UCSC genome see text footnote 1 by Hisat2 (v2.1.0) with default parameters as above. For estimation of retroelement expression, “repeatMasker” track, was downloaded from UCSC genome browser in GTF, based on the genomic coordination in GTF files. Annotation file of transposable elements was extracted and made as a SAF file, which is uploaded in https://github.com/LianaLiu/single. Reads were counted using featurecounts and only the unique mapped reads with completely overlapping retrotransposon coordinates were counted. Class and family of transposable elements are also provided in the supplementary datasets (Kaul et al., 2019).

Hormone Assays

Serum anti-Mullerian hormone (AMH) levels were assayed using ELISA kit (CK-E90200, Hangzhou EastBiopharm CO., LTD.). Quality control serum, sterilized distilled water, and five series diluted standard samples for a standard curve were tested for each serum sample. The intra- and inter-assay coefficients of variability for AMH were below 8 and 12%.
Statistical Analysis
Data were analyzed by student’s t-test, χ2 test or fisher’s exact test for paired comparison, or by ANOVA and means compared by Fisher’s protected least-significant difference (PLSD) for multiple comparisons using the StatView from SAS Institute and the graphs made by GraphPad Prism 7 (GraphPad Software, San Diego, CA, United States). Kruskal-Wallis test was used to compare multiple groups using ggpubr R package. Linear regression analysis was done using GraphPad Prism 7. The data between groups were considered significant when $P < 0.05$ (*), 0.01(**) or 0.001(***)

RESULTS

Tet1 Deficiency Reduces Follicle Reserve and Causes Premature Ovarian Failure and Infertility
To study whether Tet1 regulates fertility with age, we employed Tet1 deficient mice generated using homologous recombination by deleting exons 11-13 (Zhang et al., 2013). Homozygous Tet1 knockout mice (Tet1−/−) were produced by crossing heterozygous mice (Supplementary Figure 1A). However, Tet1 deficiency resulted in death of a small proportion of embryos (Supplementary Figure 1B), consistent with previous study (Yamaguchi et al., 2012). The Tet1 knockout was evaluated based on genotyping, as well as the minimal mRNA expression level of Tet1 determined by qPCR (Supplementary Figure 1C). Also, Tet1 protein was absent in Tet1−/− blastocysts by immunofluorescence microscopy (Supplementary Figure 1D), further validating the successful knockout of the Tet1 gene. Representative primordial oocytes were shown by enclosed granulosa cells (Supplementary Figure 1E). The 5 hmC levels of primordial oocytes from Tet1−/− mice as shown by immunofluorescence were decreased compared to WT oocytes (Supplementary Figure 1F).

Wild-type (WT) and Tet1−/− female mice at the reproductive age of 2–4 months (young), 7–9 months (middle-age) and 10–12 months (old), were mated with young WT males, and the successfully mated females judged by the presence of mating plug next morning were allowed to deliver the pups to determine the fertility. WT mice exhibited reduced fertility with age as shown by smaller litter size, as expected for natural reproductive aging in females. Comparatively, Tet1−/− female mice manifested declined fertility and accelerated reproductive failure with age, as they produced smaller litter size than that of age-matched WT mice (Figure 1A). By linear regression analysis, WT and Tet1−/− mice with age displayed similar negative slope in the production of the offspring (Figure 1B). While WT females at middle-age produced an average of six pups, Tet1−/− deficient age-matched mice or at an older age failed to give birth to a pup (Figure 1A). Tet1−/− female mice were infertile at middle-age. This phenomenon is comparable to premature ovarian failure. Consistently, ovaries of Tet1−/− females at three age groups were lighter than those of age-matched WT females from young to old age (Figure 1C). By examining the ovarian serial sections by histology, the number of primordial and primary follicles as well as secondary and mature antral follicles was dramatically reduced in Tet1+/− females from the three age groups, compared to WT mice served as control (Figures 1D,E). Slope in the declined number of follicles in WT mice with age appeared to be much higher than did Tet1−/− mice, and the decrease of secondary and antral follicles in Tet1−/− mice with age actually was not significant (Figure 1F). This likely was because the follicle reserve was already dramatically lower in Tet1−/− than in WT mice at young age. Consistently, lower AMH levels indicative of ovarian reserve were found in serum of Tet1−/− than in WT mice at young age (Figure 1G). Therefore, Tet1 deficiency decreases fecundity particularly with age. The decreased fecundity is largely caused by prominent reduction of follicle reserve and development, and also probably by reduced oocyte quality.

Transcriptome Analysis of Tet1-Deficient Oocytes in Comparison With WT Oocytes
To examine potential molecular changes in the oocytes, we performed single-cell RNA-seq detecting an average number of genes more than 15,000 (Supplementary Figure 2A) to analyze the oocytes of Tet1−/− n = 15 and WT n = 19) collected from young mice in two biological repeated experiments. Tet1−/− oocytes showed 2,013 downregulated (p value < 0.05, and a fold change < −1.5) and 1,557 upregulated genes (p value < 0.05, and a fold change > 1.5), compared with WT oocytes (Figure 2A). Significantly downregulated genes in Tet1−/− oocytes included Fmr1 and Tet1, supporting an effective deletion of Tet1. Also, we analyzed differential gene expression among chromosomes (Supplementary Figure 2A). Notably, gene expression on chromosome X (Figure 2B) and in mitochondrial functions (Figure 2C) was significantly decreased in Tet1−/− oocytes compared with WT oocytes. About 39 genes located on X-chromosomes such as Nuidj11, Tmen47, and Fmr1 were down-regulated in Tet1−/− oocytes. Due to the important roles of Fmr1, we used metascape to analyzed the potential protein-protein interaction enrichment analysis and found Fmr1 might interact with Hnrnpu and Pabpc1 (Supplementary Figure 2B). Moreover, the upregulated genes by Tet1 deficiency were enriched in homologous recombination, cell cycle, fanconi anemia pathway, Alzheimer’s disease, and metabolic pathways, while downregulated genes enriched in Ras signaling pathway, neurotrophin signaling pathway, which can cause premature ovarian failure (Dorfman et al., 2014), and notably autophagy and ubiquitin mediated proteolysis (Figures 2D–F). By GO enrichment analysis, downregulated genes after Tet1 deficiency were enriched in mRNA processing, regulation of mRNA stability, histone modification, negative regulation of apoptotic pathway such as Uru1, Rtkn2, Nrr4a2, Bcl2l2, Akt1 and Psmc3, and also protein polyubiquitination (Figures 2G,H). Interestingly, genes for DNA repair, nuclear division, chromosome segregation and organelle fission were upregulated in Tet1-deficient oocytes (Figures 2G,I). Increased apoptosis has been implicated in reduction of germ cell numbers (Yamaguchi et al., 2012). Together, these results suggest that elevated organelle fission and reduced protein polyubiquitination.
FIGURE 1 | Tet1 knockout causes subfertility and premature ovarian failure. (A) Average litter size of wild-type (WT) and Tet1 knockout (Tet1−/−) female mice at three different reproductive age (young, 2–4 months; middle-age, 7–9 months; old, 10–12 months). n > 10 mice for young and middle-age group and n = 7–8 mice for old group. (B) Linear regression analysis of the age effects on litter size between WT and Tet1−/− mice. n > 10 mice for young and middle-age group and n = 7–8 mice for old group. (C) Average weight per ovary from WT and Tet1−/− mice. n > 7. (D) Representative images of hematoxylin and eosin (H&E) staining of ovarian sections from young, middle-age and old groups. Scale bar, 500 µm. (E) Number of primordial and primary follicles, or secondary and antral follicles from WT and Tet1−/− mice. n = 4-6. (F) Linear regression analysis of the age effects on the number of follicles between WT and Tet1−/− mice. n = 3-4. ns, no significant difference. (G) AMH levels in serum of WT and Tet1−/− mice by ELISA assay (n = 4). The bars show mean ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001. The P value is labeled if P > 0.05 (A,E).

and autophagy might be implicated in reduced oocyte quality resulting from loss of Tet1.

Further, we compared our single cell RNA-seq data on oocytes with published data on PGCs (Yamaguchi et al., 2012) and found that 63 genes including Rnf8, Tet3, Fmr1Ln, and Xlr5a/b/c were commonly downregulated in both Tet1-deficient oocytes and PGCs (Supplementary Figures 3A,B). We also compared our data using oocytes with the differentially expressed genes (DEG) associated with differential methylated regions (DMR) from published data using PGCs (Yamaguchi et al., 2012). About 20 genes, including Tet1, Sycp3, Dazl, and Sirt6, exhibited reduced expression in association with increased methylation after Tet1 deficiency (Supplementary Figure 3C). The analysis shows that Tet1 also plays a role in maturing oocytes in addition to its function in PGCs and meiosis.

Transcriptome Analysis of Old Tet1-Deficient Oocytes

We further analyzed differential gene expression between old WT (n = 19) and Tet1−/− oocytes (n = 14) in two biological repeats. Tet1, as expected, and Dazl were downregulated in old Tet1−/− oocytes compared with old WT oocytes, while Dnmt3a was highly expressed in old Tet1−/− oocytes (Figure 3A). Downregulated genes in old Tet1−/− oocytes were enriched in insulin signaling pathway, Parkinson’s disease, pathways
in cancer and sphingolipid signaling pathway, and genes upregulated in old Tet1−/− oocytes enriched in spliceosome, choline metabolism in cancer, and phospholipase D signaling pathway (Figure 3B). The expression of genes enriched in insulin signaling pathway (Figure 3C), were decreased and genes enriched in spliceosome (Figure 3D) were increased in Tet1−/− oocytes. By GO enrichment analysis, genes upregulated in old Tet1−/− oocytes were enriched in cellular component disassembly, negative regulation of organelle organization, small GTPase mediated signal transduction, DNA repair, regulation of DNA metabolic process and protein localization to Golgi apparatus, whereas genes downregulated enriched in organelle
fission, protein dephosphorylation, proteasomal protein catabolic process and negative regulation of apoptotic pathway including genes including Akt1, Bcl2, Nr4a2, etc (Figure 3E). Expression of genes enriched in proteasomal protein catabolic process were decreased (Figure 3F), and those in DNA repair were increased (Figure 3G). Implication of increased DNA repair is not known, but possibly suggests increased DNA damage stress due to loss of Tet1.
Additionally, we analyzed the differential gene expression between young and old Tet1−/− oocytes to explore the aging effect on Tet1-deficiency. There were 1,117 upregulated and 1,216 downregulated genes in old Tet1−/− mouse oocytes compared with young Tet1−/− oocytes (Figure 4A). Comparatively, more genes were upregulated and downregulated in old WT oocytes (Figure 4B). By comparing the differential gene number between WT and Tet1−/− oocytes with mouse age, interestingly, aging affects gene expression more in WT than in Tet1−/− oocytes (Figure 4C). However, the mismatch repair was increased in old Tet1−/− oocytes compared with young Tet1−/− oocytes by KEGG enrichment analysis (Figure 4D), while apoptotic process was enriched in young Tet1−/− oocytes (Figure 4E). These results show that Tet1 deficiency may overlap some of the aging effect on gene expression. Alternatively, based on complex analysis above, Tet1 deficiency induces partial aging effects on oocyte function.

**Increase of LINE1 Transcripts in Tet1−/− Oocytes**

Transposable elements (TEs) constitute approximately 40% of the mouse genome (Bourc’his and Bestor, 2004) and DNA methylation plays roles in repressing TEs (Wu and Zhang, 2010; Cardelli, 2018). Previously, loss of Tet1 was associated with increased methylation and reduced transcriptional activation of TEs in PGCs (Hill et al., 2018). We analyzed transcripts of TEs in oocytes of young and old mice based on our single cell RNA-seq data. Proportion of LINE and SINE was slightly higher in Tet1−/− oocytes compared with young WT oocytes.
Further analysis of different ERVs revealed that the proportion of ERVL-MaLR was higher in Tet1−/− than that in WT oocytes (Figure 5B). Moreover, we used z-score to compare the expression of ERVL-MaLR belonging to ERVL-Ma, and showed that it was increased in Tet1−/− oocytes (Figure 5C). To further analyze the difference between young Tet1−/− and WT oocytes, we performed differential analysis using Deseq2 and found that L1-mus1 and L1-mus3 transcripts were increased in young Tet1−/− oocytes compared with age-matched WT oocytes (Figure 5D). Based on previous study, only 1/10 L1 elements were full-length with lower coding for retrotranspositionally competent L1 elements (Deininger et al., 2017). We further
analyzed the full-length L1 family and found that L1Md_F3 was notably highly expressed in Tet1−/− deficient oocytes, in contrast to WT oocytes (Figure 5E). These results together suggest that Tet1 deficiency also may alter transcription of TEs in oocytes.

**DISCUSSION**

Here, we show that Tet1 deficiency leads to POF by influencing the quality of oocytes, including aberrant X-chromosome inactivation, and increased expression of L1-mus, as well as the oocyte number and follicle reserve. Previous elegant study demonstrated critical role of Tet1 in meiosis/PGCs during fetal development (Yamaguchi et al., 2012). Tet1 deficiency leads to aberrant meiosis, increased methylation on meiosis genes and subfertility in young mice (Yamaguchi et al., 2012). We extended the study by evaluating age effects on the fertility and oocytes in adult mice deficient in Tet1. We find that Tet1 deficient mice at reproductive middle-age already are completely infertile, consistent with POF or primary ovarian insufficiency (POI), which is characterized by the premature depletion of ovarian follicles and infertility at mid-reproductive age (Shah and Nagarajan, 2014; Delcour et al., 2019).

We show that Tet1 deficiency upregulates genes for Alzheimer's diseases, but reduces expression of X-chromosome-linked genes, such as Fmr1. These defects might be linked in POF. Women with primary ovarian insufficiency (POI) or POF are at increased risk for non-communicable diseases such as, Alzheimer's disease, cardiovascular disease, and osteoporosis (Harlow and Signorello, 2000; Shah and Nagarajan, 2014). POF has repeatedly been associated to X-chromosome deletions and haploinsufficiency of X-linked genes can be on the basis of POF (Ferreira et al., 2010). X chromosome inactivation could underlie POF linked to cognitive impairment in Alzheimer's disease (Al-Hinti et al., 2007; Bretherick et al., 2007; Davey, 2013), regardless existing discrepancy on X chromosome in POF. Moreover, fragile X mental retardation type 1 (FMR1) gene premutation on the X chromosome have been frequently found in POF or POI (Jin et al., 2013; Bretherick et al., 2007; Davey, 2013), likely reducing expression of FMR1. Indeed, blocking DNA methylation by 5-azacytidine (5-azaC or 5-azadC) has achieved a significant reactivation of FMR1 gene expression in fragile X syndrome cellular models (Bar-Nur et al., 2012). Also, L1 and ERVL-MaLR are aberrantly expressed in Tet1-deficient oocytes. L1 is highly expressed during mouse oocyte development and embryo cleavage stage (Jachowicz et al., 2017). But, elevated L1 expression correlates with fetal oocyte attrition, oocyte aneuploidy and embryonic lethality (Malki et al., 2014).

Additionally, Tet1 deficiency results in declined ubiquitination and autophagy but increased organele fission in oocytes, and these defects presumably can be detrimental to clearance of damaged, or, senescent organelles in the cells. Impaired autophagy can lead to POF (Gawriluk et al., 2014; Delcour et al., 2019). Tet1 deficiency also decreases Ras signaling pathways. Appropriate activation of RAS signaling is crucial for directing normal follicle development (Fan et al., 2008).

Further experiments are required to investigate the underlying molecular mechanisms of how Tet1 regulates these signaling pathways associated with POF or POI. Also, the question remains as to whether Tet1 has similar function in human fertility and in POF patients. Nevertheless, our findings that Tet1 may involve in autophagy, X-chromosome activation and Alzheimer's disease provide additional insights into molecular basis of POF.

**DATA AVAILABILITY STATEMENT**

RNA-seq data have been deposited in the GEO database under the accession number GSE142163.

**ETHICS STATEMENT**

The animal study was reviewed and approved by the Institutional Animal Care and Use Committee, Nankai University. Written informed consent was obtained from the owners for the participation of their animals in this study.

**AUTHOR CONTRIBUTIONS**

HW performed the experiments on fertility and histology, and prepared the manuscript. LinLiLiu analyzed the RNA-seq data and prepared the manuscript. G_LX provided the materials, advised the project, and revised the manuscript. HW performed the experiments on fertility and histology, and prepared the manuscript. LinLiLiu analyzed the RNA-seq data and prepared the manuscript. G_LX provided the materials, advised the project, and revised the manuscript. HW performed the experiments on fertility and histology, and prepared the manuscript. LinLiLiu analyzed the RNA-seq data and prepared the manuscript. G_LX provided the materials, advised the project, and revised the manuscript. HW performed the experiments on fertility and histology, and prepared the manuscript. LinLiLiu analyzed the RNA-seq data and prepared the manuscript. G_LX provided the materials, advised the project, and revised the manuscript. HW performed the experiments on fertility and histology, and prepared the manuscript. LinLiLiu analyzed the RNA-seq data and prepared the manuscript. G_LX provided the materials, advised the project, and revised the manuscript. HW performed the experiments on fertility and histology, and prepared the manuscript. LinLiLiu analyzed the RNA-seq data and prepared the manuscript. G_LX provided the materials, advised the project, and revised the manuscript. HW performed the experiments on fertility and histology, and prepared the manuscript. LinLiLiu analyzed the RNA-seq data and prepared the manuscript. G_LX provided the materials, advised the project, and revised the manuscript. HW performed the experiments on fertility and histology, and prepared the manuscript. LinLiLiu analyzed the RNA-seq data and prepared the manuscript. G_LX provided the materials, advised the project, and revised the manuscript. HW performed the experiments on fertility and histology, and prepared the manuscript. LinLiLiu analyzed the RNA-seq data and prepared the manuscript. G_LX provided the materials, advised the project, and revised the manuscript. HW performed the experiments on fertility and histology, and prepared the manuscript. LinLiLiu analyzed the RNA-seq data and prepared the manuscript. G_LX provided the materials, advised the project, and revised the manuscript.
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SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2021.644135/full#supplementary-material

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![Supplementary Table 2](https://www.frontiersin.org/articles/10.3389/fcell.2021.644135/full#supplementary-material)

**Supplementary Table 2.** (B) Table summarizing the litter size and Mendelian ratio of Tet1−/− mice. (C) Tet1 mRNA expression level in ovaries of young mice by qPCR analysis. (D) Tet1 protein expression level in E3.5 blastocysts by immunofluorescence. Scale bar, 10 μm. (E) Representative images of primordial follicles from young WT mouse ovary. (F) Representative images of primordial follicles stained with 5 hmC antibody. Dashed line indicates the primordial oocyte. Scale bar, 10 μm. Bottom panel, Relative 5 hmC level in primordial oocytes from young mice. n = 21 oocytes counted for each group. Data represents mean ± SEM. ***P < 0.001.

**Supplementary Figure 2** | Distribution of differentially expressed genes across chromosomes. (A) Scatter plot illustrating the chromosomal distribution of differential genes Between young WT and Tet1−/− oocytes. (B) Protein-protein interactome network analysis using Metascape (http://metascape.org/gp/index.html) showing the potential interaction of Fmr1, Hnrnpu and Pabpc1 and with other proteins.

**Supplementary Figure 3** | Coverage of Smart-seq2 of oocytes and comparison with transcriptome data on PGCs from Yamaguchi et al., 2012. (A) Boxplot displaying number of genes sequenced in oocytes. (B) Venn plot showing the overlapped, downregulated genes between oocytes based on our data and PGCs from Yamaguchi et al., 2012. (C) Venn plot showing the overlapped, downregulated genes between oocytes and PGCs associated with DMRs, based on Yamaguchi et al., 2012.
Liu et al. Role of Tet1 in Fertility

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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