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RAPID COMMUNICATION

Increased N6-methyladenosine is related to the promotion of the methyltransferase METTL14 in ovarian aging

The ovary is a vital female reproductive organ that functions to produce oocyte gametes and cyclically expressed sex hormones to maintain reproductive capacity and hormone homeostasis. Ovarian aging, characterized by declines in follicle quantity and oocyte quality, leads to premature ovarian failure, premature ovarian insufficiency, or diminished ovarian reserve, thus contributing to female infertility. Distinct gene-expression signatures from RNA transcriptional level and post-transcriptional regulation are relative to oocyte quality and reproductive age.[1] Mouse follicular transcriptome-wide N6-methyladenosine (m^6A) landscape revealed that dynamic m^6A modification exists in the process of maternal-to-zygotic transition, which depended on the regulation by the m^6A methyltransferase complex.[2] m^6A writing mediated by maternal methyltransferase-like 3 (METTL3) is a key role in the establishment of m^6A on oogenesis and pre-implantation embryo development.[2] Methyltransferase-like 14 (METTL14) serves as the core subunits to catalyze effective methyl group transfer and is an important role in promoting the aging-associated phenotype and reprogramming.[3] However, the regulatory mechanisms from m^6A association complements during the process of ovarian aging remain incompletely explored.

To explore whether mRNA m^6A modification takes part in the potential mechanism of aging in human ovaries, we first employed an immunohistochemistry assay to evaluate the content of m^6A modification in normal human ovaries from young-, middle-, and old-aged women. The level of m^6A modification was elevated significantly in aged human ovaries compared with young ovaries (Fig. 1A,B). In addition, human granulosa cells (hGCs) of healthy women from 24 to 42 years old were obtained. Subsequently, m^6A-specific dot blotting was executed to measure RNA methylation levels. Compared with the young age group, the dot blot analysis revealed a clear up-regulation in the old age group (Fig. 1C,D). To further determine whether m^6A modification-related writers may contribute to these elevated m^6A levels, we found that m^6A methyltransferase METTL14 expression was increased in the ovaries of the old age group compared with those in the young age group (Fig. 1E,F). The mRNA levels of *METTL14* in the hGCs were evaluated by reverse transcription quantitative real-time PCR (RT-qPCR). As shown in Figure S1, the mRNA level of *METTL14* in the old age group was markedly higher than that in the young age group.

To determine whether METTL14 is similarly up-regulated in aged mouse ovaries, the ovaries of young-, middle-, and old-aged mice were evaluated. The ovaries in the old age group were clearly small (Fig. 1G). Similarly, the ovarian weight was obviously decreased in the old age group in comparison to that in the young age group (Fig. 1H). As shown in Figure S2A, hematoxylin and eosin (H&E) staining was used to count the four stages of ovarian follicles. A more than ten-fold decrease in primary follicles was observed in the old age group versus the young age group (Fig. S2B), along with a more than three-fold decrease in secondary follicles in the old age group compared with the young age group (Fig. S2C). A similar trend was observed in the total follicle number (Fig. S2D). Consistent with the results in human ovaries, Western blot (WB) showed that the protein level of METTL14 was apparently elevated in old-aged mouse ovaries (Fig. 1I,J). Collectively, these results suggest that higher METTL14 expression level promotes ovarian aging in mice. In addition, high passage hGCs were employed to confirm the m^6A level and METTL14 protein level. The results revealed that a high passage culture process could prompt the m^6A level and the protein level of METTL14 (Fig. S3A–D).

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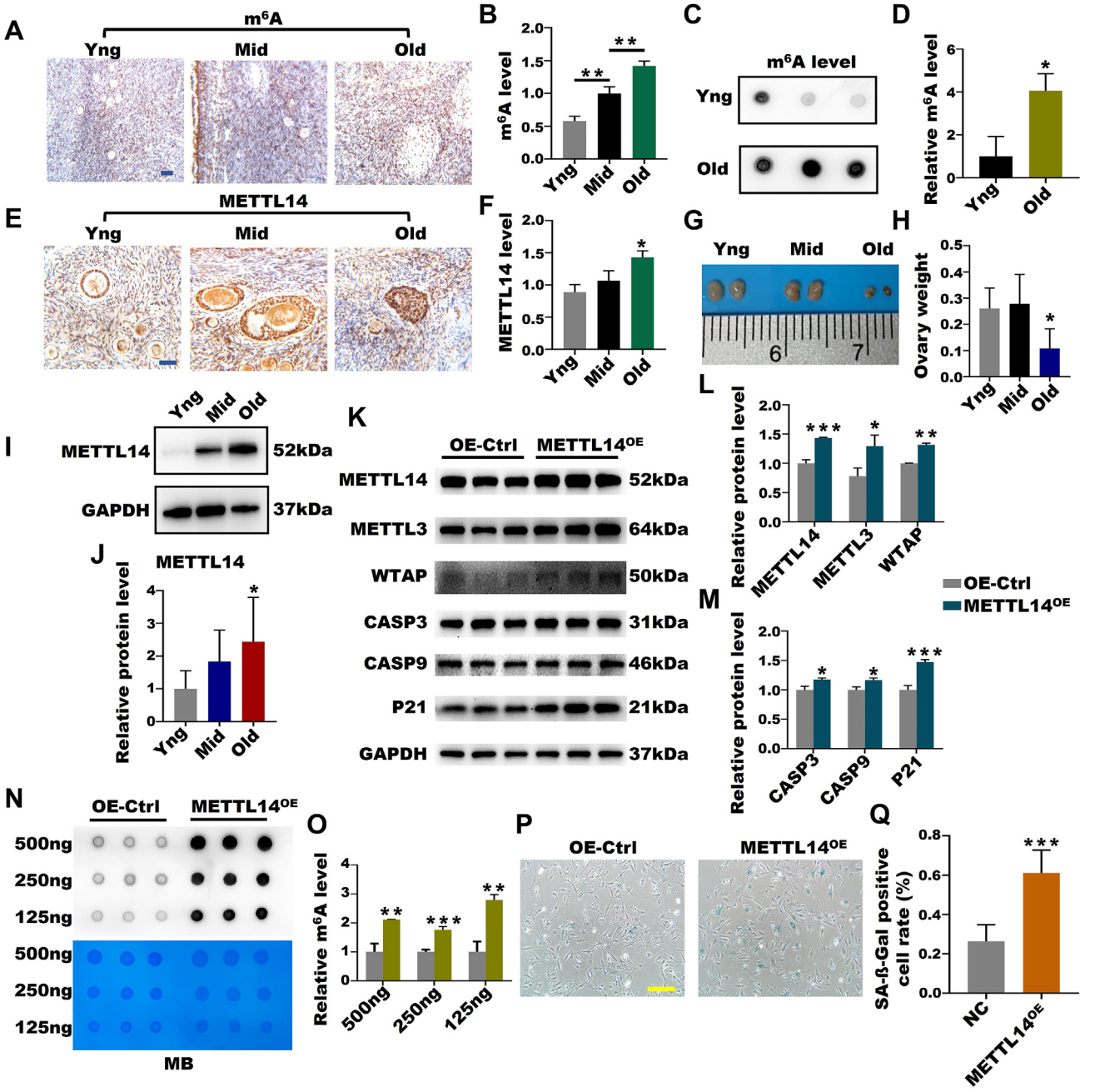

Figure 1 Overexpression of METTL14-induced ovarian aging. **(A)** Immunohistochemical (IHC) microscopy image of m^{6}A expression in human ovaries from the young (Yng), middle (Mid), and old age groups. Scale bar, 50 μm. **(B)** IHC analysis showed up-regulation of the IHC score of m^{6}A expression in old-aged ovaries compared with young-aged ovaries. $^{**}P < 0.01$. **(C)** Dot blot image of m^{6}A expression in hGCs from the young and old age groups. **(D)** Dot blot analysis showed the up-regulation of m^{6}A level in old-aged hGCs compared with young-aged hGCs. $^{*}P < 0.05$. **(E)** IHC microscopy image of METTL14 expression in human ovaries from the young (Yng), middle (Mid), and old age groups. Scale bar, 50 μm. **(F)** IHC analysis showed up-regulation of the IHC score of METTL14 expression in old-aged ovaries compared with young-aged ovaries. $^{*}P < 0.05$. **(G)** Phenotype images in mouse ovaries from the young, middle, and old age groups. **(H)** Mouse ovarian weight analysis showed down-regulation in the old age group compared with the young age group. $^{*}P < 0.05$. **(I)** WB image of METTL14 expression in mouse ovaries from the young, middle, and old age groups. **(J)** WB analysis showed up-regulation of METTL14 protein levels in old-aged mouse ovaries in comparison to young-aged mouse ovaries. $^{*}P < 0.05$. **(K)** WB image of m^{6}A methyltransferase complex (METTL14, METTL3, WTAP), apoptosis genes (CASP3, CASP9), and aging gene (P21) in METTL14^{OE} cells. **(L, M)** WB analysis showed up-regulation of the m^{6}A methyltransferase complexes (METTL14, METTL3, WTAP), apoptosis genes (CASP3, CASP9), and aging gene (P21) in METTL14^{OE} cells. OE-Ctrl, overexpression-control. **(N)** Dot blot image of m^{6}A expression in METTL14^{OE} cells. MB, methylene blue; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. **(O)** Dot blot analysis showing the upregulation of m^{6}A level in METTL14^{OE} cells. $^{**}P < 0.01$, $^{***}P < 0.001$. **(P)** Microscopy image of SA-β-Gal-positive cells in the OE-Ctrl and METTL14^{OE} groups. Scale bar, 200 μm. **(Q)** SA-β-Gal activity analysis showed up-regulation of the percentage in SA-β-Gal-positive cells in the METTL14^{OE} group. NC, negative control. $^{***}P < 0.001$.

Given that METTL14 is up-regulated in ovaries during ovarian aging in both humans and mice, we explored the function of METTL14 overexpression in KGN cells (METTL14^{OE}). RT-qPCR was employed to verify the knock-in efficiency, and the mRNA level was significantly increased in KGN cells after transfection with the METTL14 plasmid (Fig. S3E). Similarly, the protein level of METTL14 was found to be increased in METTL14^{OE} cells detected by WB (Fig. 1K). Besides, the protein level of other m^{6}A methyltransferase complexes (METTL3, WTAP) was elevated by METTL14^{OE} (Fig. 1L). Apoptotic inducer (CASP9) and apoptotic executor (CASP3) were promoted after METTL14 overexpression (Fig. 1M). Our results found that the aging gene P21 protein level was increased in the METTL14^{OE} cell line (Fig. 1M). In addition, overexpression of METTL14 resulted in up-regulation of the m^{6}A modification level in METTL14^{OE} cells compared with that in OE-Ctrl (overexpression-control) cells, as shown by m^{6}A dot blot analysis (Fig. 1N,O). Furthermore, overexpression of METTL14 apparently increased the percentage of SA-β-Gal-positive cells (Fig. 1P,Q). Proliferative capacity was measured by CCK-8 assay, and the results indicated that it was markedly down-regulated in METTL14^{OE} cells after 72 h and 96 h of transfection (Fig. S4).

METTL14 and METTL3 are the core subunits of the m^{6}A methyltransferase complex and exhibit catalytic activity in regulating RNA stability, maturation, and export during oogenesis and ovarian development.[2] A previous study investigated whether m^{6}A peaks and m^{6}A modification increased from 6 weeks to 52 weeks in four regions of the mouse brain and from adolescence to older age in the human brain.[4] These findings suggested that m^{6}A plays a regulatory role during senescence or premature senility. Interestingly, a recent study investigated that METTL14 and METTL3 contributed to an m^{6}A-independent senescence-associated secretory phenotype in addition to increased m^{6}A in senescent primary human lung embryonic fibroblasts induced by oncogenic RAS.[5] This difference may be attributed to organ or cell heterogeneity during the aging process. Whereas the degree of METTL14's influence on ovarian aging is undiscovered. Our study proposes a novel model in which m^{6}A modification driven by METTL14 accelerates cell senescence and inhibits cell proliferation to induce ovarian aging. This evidence connecting m^{6}A and METTL14 provides a potential molecular mechanism to regulate ovarian aging at an epigenetic level. Further rigorous work to explore the targets of METTL14 is needed to achieve a complete understanding of this mechanism.

Ethics declaration

The use of human ovarian granular cells and ovarian tissue section were in accordance with the relevant guidelines and regulations and the experimental protocols were approved by the Medical Ethics Committee of the Suzhou Hospital Affiliated to Nanjing Medical University (2020322). All the patients provided written informed consent prior to participation in this study. Our investigation using experimental animals was conducted following the Nanjing Medical University Animal Center's specific guidelines and standards (20170480).

Author contributions

B.H. and H.L. conceptualized this project and wrote and reviewed this manuscript. C.Q. and Z.L. conducted functional experiments. H.S. picked suitable human ovaries paraffin samples and analyzed the data. J.D. and H.S. collected and isolated hGCs. R.C. and M.S. performed H&E and IHC assay. All authors read and agreed to the published version of the manuscript.

Conflict of interests

The authors declare no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.06.019.

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