

Letter to the Editor

Spatiotemporal Transcriptome Analysis Reveals Activation of the AP1 Pathway in the Ovarian Microenvironment during the Transition from Premenopause to Postmenopause

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[Received April 3, 2023; Revised June 6, 2023; Accepted July 7, 2023]

Dear Editor,

Although life expectancy in developed countries has increased markedly from 45 to 85 years over the past two centuries, the age at natural menopause (ANM) of healthy women has remained relatively stable (average 50.5, 47-53 years) [1]. Thus, there can be a substantial gap between reproductive ageing and somatic ageing. Factors other than overall lifespan, such as genetics, play a significant role in determining ANM [1, 2]. It is well known that menopause is the end of a woman's reproductive period and menstrual cycles. However, the molecular basis of the transition from premenopause to postmenopause in the ovaries remains to be determined, especially with respect to the changes in gene expression and cell type components. Herein, we performed spatial transcriptomic sequencing of human pre- and postmenopausal ovaries (n=4, 32-56 years), linked with single-cell transcriptomic analysis of ovaries from young (n=4, 4-5 years) and old (n=4, 18-20 years) nonhuman primates (NHPs) [3], to characterize the changes in expression in human ovaries

from premenopause to postmenopause with spatial and single-cell resolution (Fig. 1A).

After quality control, we retained 13,091 spatial transcriptomic data spots (Supplementary Table 1). Next, single-cell transcriptomic data [3] from 7 cell types (oocyte, granulosa cell, stromal cell, natural killer T (NKT) cell, macrophage, endothelial cell, and smooth muscle cell (SMC), Supplementary Fig. 1A, and <https://doi.org/10.7910/DVN/D8RZKK>) were used to annotate the spatial transcriptomic spots with Cell2location [4] (a Bayesian model), and 22 clusters were identified. The results revealed that the location of spot clusters and gene expression abundance were associated with the histological structure of the ovary, from the ovarian cortex to the medulla (Fig. 1B-C, Supplementary Fig. 1B-D). We then estimated the cell abundances of 7 cell types across ovarian regions, and the results demonstrated that our model provided sufficient sensitivity and resolution to spatially map the cell types (Fig. 1D).

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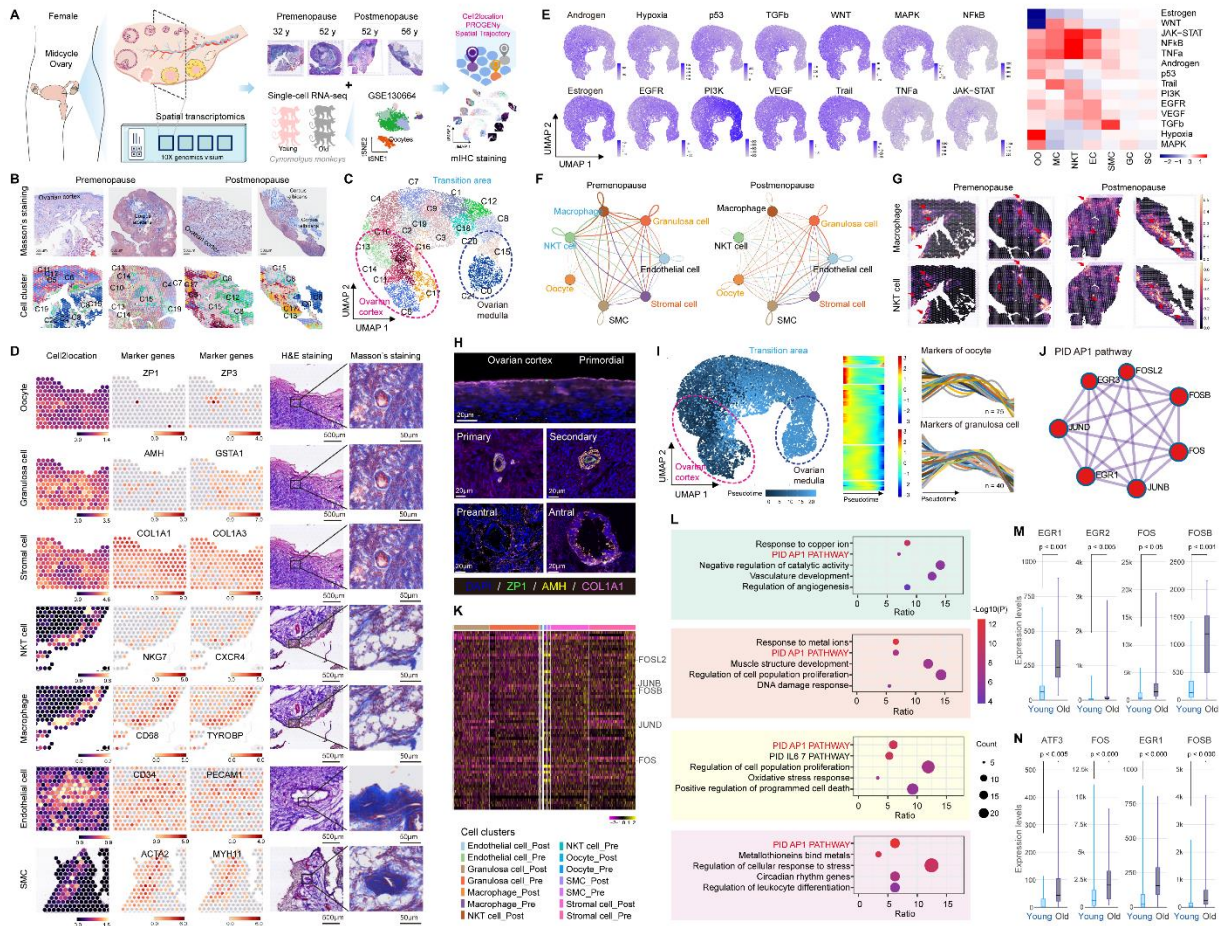


Figure 1. Spatiotemporal characteristics of human ovaries during the transition from premenopause to postmenopause. (A) Study flowchart of sequencing and analysis. (B) Masson’s staining of samples and spot cluster spatial transcriptomics data via Cell2location. (C) UMAP plot of spatial transcriptomic spots. Each colour represents a cluster. (D) Spatial transcriptomic spot cell type predictions, marker gene expression, H&E staining, and Masson’s staining of the corresponding area. The scale colour of Cell2location analysis establishes the spatial mapping of the cell types. (E) PROGENy results of signalling pathways in the UMAP plot and cell types. (F) Cell communication analysis among cell types via CellPhoneDB. The nodes represent cell types and are distinguished by different colours. Each line between nodes represents the relationship of cell type A with cell type B. The node with the same colour as the line represents the beginning, and the other node represents the end. The thickness of the line represents the number of ligand–receptor pairs. (G) Immune cell distribution at spatial resolution via Cell2location analysis. (H) fm-IHC results of the different stages of follicles and stromal cells. (I) Pseudotime trajectory analysis of all spatial spots by Monocle2. The UMAP plot of spatial spots is coloured by pseudotime. The heatmap shows the gene expression patterns in each cluster along the pseudotime trajectory, coloured by gene expression levels. The colour intensity indicates the average gene expression. Expression changes in marker genes of oocytes and granulosa cells across the pseudotime trajectory are shown in the scatter plot. (J) Main components of the AP1 pathway. (K) Heatmap of the cell types in the ovaries between premenopause and postmenopause. (L) Representative pathway and process enrichment terms for upregulated genes of postmenopause vs. premenopause in stromal cells, SMCs, NKT cells and macrophages (from top to bottom). AP1 pathway-associated genes upregulated in macrophages (M) and NKT cells (N) in old NHP ovaries at the single-cell transcriptomic level.

We then used the PROGENy algorithm [5] and found that signalling pathways were activated in different structures or cell types, such as the PI3K pathway in the ovarian medulla and the oestrogen pathway in postmenopausal ovaries and granulosa cells (Fig. 1E, Supplementary Fig. 1E-G). Altered intercellular communication is one of the hallmarks of human ageing [6]. In this study, the total number and overall strength of

intercellular interactions of cell types at spatial levels were reduced in postmenopausal samples (Fig. 1F). Of note, cell interactions between stromal cells and granulosa/immune cells were significantly decreased (Supplementary Fig. 2A-F). To explore potential mechanisms underlying the changes, we compared the cell distribution and found that macrophages colocalized with NKT cells (Fig. 1G, Supplementary Fig. 3A-B), with

higher abundances at premenopause (Supplementary Fig. 3C-E), which was the same as the trend observed in NHP and mouse ovaries (Supplementary Fig. 3F-H). For stromal cells, strong colocalization signals with oocytes and granulosa cells were found and validated (Fig. 1H, Supplementary Fig. 3A-B), and the expression levels increased at premenopause (Supplementary Fig. 4A-B). In contrast, the expression levels of SMCs were decreased at premenopause (Supplementary Fig. 4A), indicating stronger angiogenesis after menopause. The differential activation patterns of stromal cells and SMCs were validated in human ovaries and were also identified in NHP ovaries (Supplementary Fig. 4C-E).

By performing spatial trajectory analysis, we found that the pseudotime trajectory of the spots was initially distributed in the ovarian cortex and finally in the late transition area and ovarian medulla (Fig. 1I). Furthermore, the expression of oocytes and granulosa cells changed along the pseudotime trajectory (Fig. 1I), which is consistent with the follicle development process from the ovarian cortex to the medulla area. The gene expression profiles along the pseudotime trajectory were enriched in processes related to response to hormone or stress and showed different expression patterns of the AP1 pathway among ovarian regions (Supplementary Fig. 5A-B, and <https://doi.org/10.7910/DVN/D8RZKK>). The AP1 pathway is composed of several key members, such as FOS, FOSB, EGR1, and JUNB, and multiple downstream regulatory factors (Fig. 1J). Since AP1 pathway-associated genes were upregulated in the ovarian cortex and medulla (Fig. S5B), we further investigated the expression of these genes in the 7 cell types, and the results revealed that the expression of AP1 pathway-associated genes was increased in postmenopausal ovaries (Fig. 1K, Supplementary Table 2-4). Moreover, the upregulated genes of stromal cells, SMCs, NKT cells and macrophages in postmenopausal ovaries were all enriched in the PID AP1 pathway (Fig. 1L), revealing the important role of the AP1 pathway in the transition from premenopause to postmenopause. Notably, similar AP1 pathway-associated gene expression and enrichment changes were found in old NHP ovaries compared with young NHP ovaries (Fig. 1M-N, Supplementary Fig. 5C-D). When comparing the differentially expressed genes between premenopause and postmenopause in oocytes and granulosa cells, activated patterns of the AP1 pathway were not observed (Supplementary Fig. 5E-G). These results indicate that the architecture and gene expression of the ovarian microenvironment are remodelled during the transition from premenopause to postmenopause and that the AP1 pathway may play an important role in both human and NHP reproductive senescence.

The ovaries are the primary sources of oestrogen in a woman's body, and the decline in their function during the

transition to menopause leads to the end of menstruation and fertility [3]. A previous study revealed dynamic changes in mechanical components and showed that collagen expression in the ovaries peaks at reproductive age [7], which is consistent with the results of this study. Indeed, the localization of follicles in the collagen-rich cortex provides an environment that supports follicle architecture and probably plays a role in follicle survival [8], while ovarian stiffness or fibrosis limits oocyte maturation and is associated with ovarian ageing [9]. Additionally, as follicles grow and ovulate and corpora lutea develop, cyclic structural changes may also affect the distribution of stromal cells [10]. However, the mechanisms of dynamic changes in the collagen component of stromal cells during the ovary transition should be further determined. AP1 is a key transcription factor regulating several cellular processes associated with cell survival proliferation and differentiation, and dysregulated expression and activity of AP1 have been implicated in several severe diseases, especially inflammatory disorders and cancer [11]. Research on the role of the AP1 pathway in ovarian development and ageing is currently limited. However, existing evidence indicates that in human ovaries, the expression of AP1 subunits is elevated in periovulatory follicles and is regulated by human chorionic gonadotropin [12]. Further studies are needed to explore the specific mechanisms by which the AP1 pathway influences ovarian function and the potential role of this pathway in ovarian ageing.

Due to the rarity of ovarian samples, the sample size of this study was small, which may have caused bias in the results and conclusions. In addition, although humans and NHPs share a high degree of genetic similarity, there are differences in gene expression between the two species. Therefore, future studies with a larger sample size and analyses of the differences between species regarding ovary ageing are needed.

Overall, this study provides a spatiotemporal perspective for clarifying the changes in the function of human ovaries during the transition from premenopause to postmenopause. During the transition, a significant shift occurs in gene expression and intercellular interactions among different cell types. Notably, the AP1 pathway becomes activated within the ovarian microenvironment. In this study, the spatial distribution of spots and clusters exhibited distinct structural characteristics, wherein the gene expression profiles along the pseudotime trajectory were enriched in processes associated with hormones or stress responses as well as the AP1 pathway. Taken together, these findings provide insights into the complex biological transition of ovary ageing with spatial and single-cell resolution.

Acknowledgements

We thank CapitalBio Technology Co., Ltd. (Beijing), for their excellent assistance with our research. We would also like to thank the groups that maintain the online tools Metascape, GEO, Aging Atlas, and The Human Protein Atlas. This work was funded by the National Key R&D Program of China (2021YFC2700303) and the National Natural Science Foundation of China (82288102, 82225019, 82192873, 81971381)

Conflicts of interest

The authors declare that they have no competing interests.

Supplementary Materials

The Supplementary data can be found online at: www.aginganddisease.org/EN/10.14336/AD.2023.0707-1.

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