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Current Opinion in
Endocrine and Metabolic Research

Review

Molecular makeup of the human adult ovary

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Abstract

Functional ovarian cells are essential for human fertility. In the adult ovary, different cell types ensure ovary homeostasis, enable hormonal production, and support oocyte maturation. Hence, the ovary is a complex and highly dynamic organ composed of a great diversity of cell types, with many still uncharacterized. The use of single-cell RNA sequencing technologies on human ovarian tissue is starting to unravel the molecular signature of the cells present in the ovary, highlighting dramatic changes in gene expression during follicular growth and regression. This knowledge will ultimately provide insights into female fertility and associated reproductive diseases and will allow the optimization of human-based disease models and in vitro gametogenesis protocols.

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Current Opinion in Endocrine and Metabolic Research 2021, **18**:187–193

This review comes from a themed issue on **Regulation of Ovarian Function**

Edited by **Norah Spears** and **Kate Hardy**

For complete overview of the section, please refer the article collection - Regulation of Ovarian Function

Available online 8 April 2021

https://doi.org/10.1016/j.coemr.2021.03.016

Keywords

Human, Adult ovary, Transcriptional signature, Single-cell sequencing, Folliculogenesis.

Abbreviations

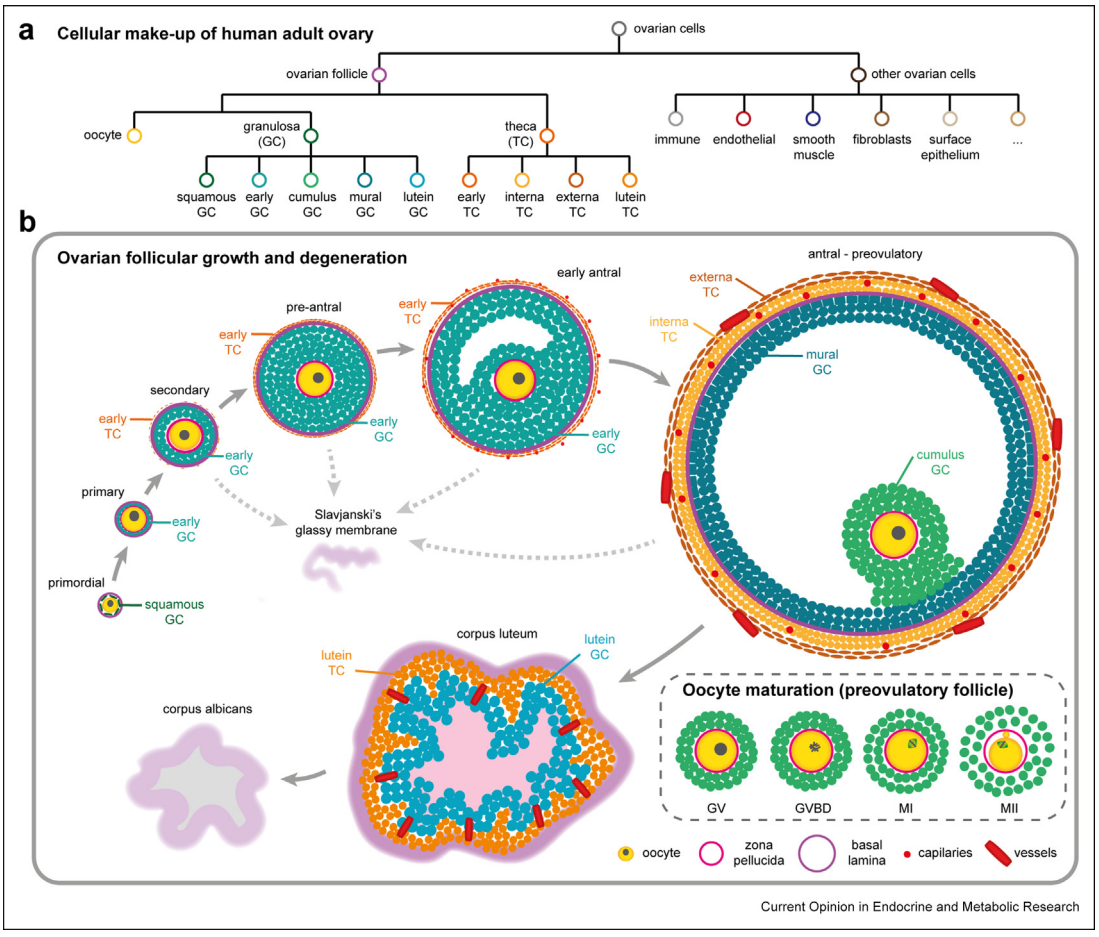
TC, theca cell; GC, granulosa cell; mm, millimeter; GV, germinal vesicle; GVBD, germinal vesicle breakdown; LH, luteinizing hormone; MI, metaphase I; MII, metaphase II; OSE, ovarian surface epithelium; COC, cumulus–oocyte complex.

Introduction

The human ovary is a highly complex and dynamic reproductive exocrine organ composed of many different cell types (Figure 1a) and responsible for storage and maturation of oocytes and cyclic production of hormones, such as progesterone and estrogen. The maturation of a primary oocyte into a competent oocyte, ready for ovulation, is a process tightly regulated by ovarian somatic cells [1–3]. Each oocyte is encapsulated by somatic cells, forming a functional unit known as the follicle. The two main somatic cell types responsible for formation and growth of the follicle are granulosa cells (GCs), surrounding the oocyte, and theca cells (TCs), aligned on the outer surface of the basal lamina [4,5]. Those two lineages undergo a dramatic development during follicular growth/maturation, followed by either regression or further development into the corpus luteum (Figure 1b). Depending on the stage of the follicle (or subsequent corpus luteum), the GC morphology can be subdivided into squamous, cuboidal, mural, cumulus, corona radiata, and lutein GCs (Figure 1). The development of the TC lineage is less well understood, but includes several well-known subtypes, such as interna, externa, and lutein TC (Figure 1). As the GC and TC develop, the follicle transits from primordial, primary, secondary (<0.15 mm), preantral (0.15–0.2 mm) (Figures 1b, 2a–d) to antral follicles (0.2–5 mm), when early GCs separate into cumulus GCs surrounding the oocyte and mural GC lining the basal lamina (Figures 1b, 2e–f). In the ovary, there are many antral follicles, but per menstrual cycle, typically one will become dominant and increase in size, reaching about 20 mm before ovulation [6]. After ovulation, that follicle transforms into the corpus luteum containing lutein GCs and lutein TCs (Figure 2g). Inevitably, the corpus luteum undergoes luteolysis and regresses into the corpus albicans [7,8], whereas most antral follicles that undergo atresia degenerate [9], leaving behind the Slavjanski's glassy membrane (basement membrane) as the only remnant (Figure 1b).

Owing to recent developments in single-cell sequencing technology, we are starting to gain in-depth knowledge on the different cell types present in the adult ovary that are responsible for follicular growth and degeneration as well as the extraordinary tissue remodeling that takes place in the human adult ovary during each menstrual cycle [10–13]. In addition, the ovary also contains many other cell types, such as cells of the ovarian surface epithelium (OSE) and stromal cells that include a variety of immune cells, endothelial cells of blood and lymph vessels, smooth muscle cells, and fibroblasts of the tunica albuginea and ovarian medulla [14] (Figure 1).

Figure 1



Main cell types in the human adult ovary. **(a)** Cartoon overview of the cellular makeup present in the human adult ovary and **(b)** the development of the oocyte, granulosa cell (GC), and theca cell (TC) during folliculogenesis. GV, germinal vesicle; GVBD, germinal vesicle breakdown; MI, metaphase I; MII, metaphase II.

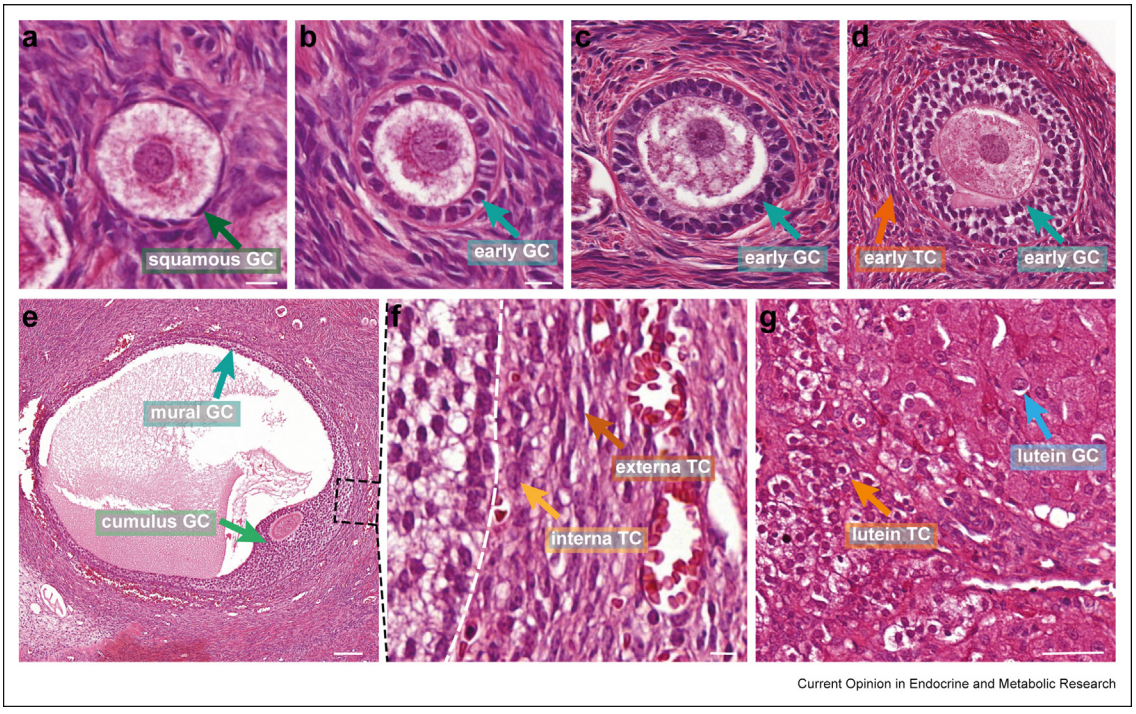
Molecular dynamics of oocytes during folliculogenesis

Primary oocytes are arrested at dictyate (diplotene of meiotic prophase I), in the so-called germinal vesicle (GV) stage, and are present in the ovarian cortex, where they constitute a finite pool of resting follicles that have not started to grow. After a surge in luteinizing hormone in the preovulatory follicle, meiotic resumption starts with germinal vesicle breakdown, but pauses once more at metaphase II (MII), until it concludes with the extrusion of the second polar body after fertilization [15] (Figure 1b). Several well-known germ cell and oocyte-specific genes, including *DDX4*, *DPPA3*, *GDF9*, *OOSP2*, *ZP2*, *ZP3*, *FIGLA*, *LHX8*, *SOHLH2*, *DAZL*, and *SOX30*, are detected across maturation stages [12,13,16–18] (See Figure 3).

Laser capture microdissection followed by transcriptional analysis has revealed substantial differential expression between human oocytes from primordial and primary follicles, such as high *FOXO1*/FOXO1 expression in oocytes in primordial follicles and high *EIF4E*/EIF4E expression in oocytes in primary follicles [19]. In addition, nuclear localization of FOXO3 and PTEN is involved in maintaining dormancy, whereas nuclear accumulation of phosphorylated Akt and phosphorylated mTOR initiates the transition from primordial to primary follicles [20]. In agreement, activation of the PI3K/Akt/mTOR pathway stimulates this transition [21]. Single-cell transcriptomics has further revealed that oocytes in primary follicles express high levels of *CLVS1*, *STAT4*, *TLR4*, *CDK14*, and *DDIT4L*, whereas oocytes in secondary follicles are enriched in *USP27X*, *PAEP*, *COX7A1*, and *CHST7*, and oocytes in early antral follicles show upregulation of *NTF4*, *LCP2*, *NSMF*, and *ABCD1* [13] (See Figure 3).

Microarray analysis of preovulatory oocytes from the GV stage to MII showed that expression of *PCNA*, *FGF14*, *BMP15*, and *CDC25A* are largely increased in MII

Figure 2



Images of granulosa cells (GCs) and theca cells (TCs) during folliculogenesis in humans. Histological images showing hematoxylin and eosin staining of **(a)** primordial follicles, **(b)** primary follicles, **(c)** secondary follicles, **(d)** preantral follicles, and **(e)** antral follicles, with **(f)** an insert magnified and **(g)** the corpus luteum showing different types of GCs and TCs. Scale bar in a–d and f is 10 μm, in e is 100 μm, and in g is 50 μm.

oocytes compared with GV and metaphase I oocytes [22]. More recently, high expression of *RBBP7* was observed in MII oocytes compared with metaphase I oocytes using single-cell transcriptomics [23]. In addition, several studies have used single-cell transcriptomics to compare the oocyte quality of patients of different ages with different reproductive disorders (endometriosis, polycystic ovary syndrome) or oocytes undergoing in vitro maturation with those of healthy females [24–32]; however, owing to the low number of available oocytes, a robust characterization of GV to MII progression is still lacking.

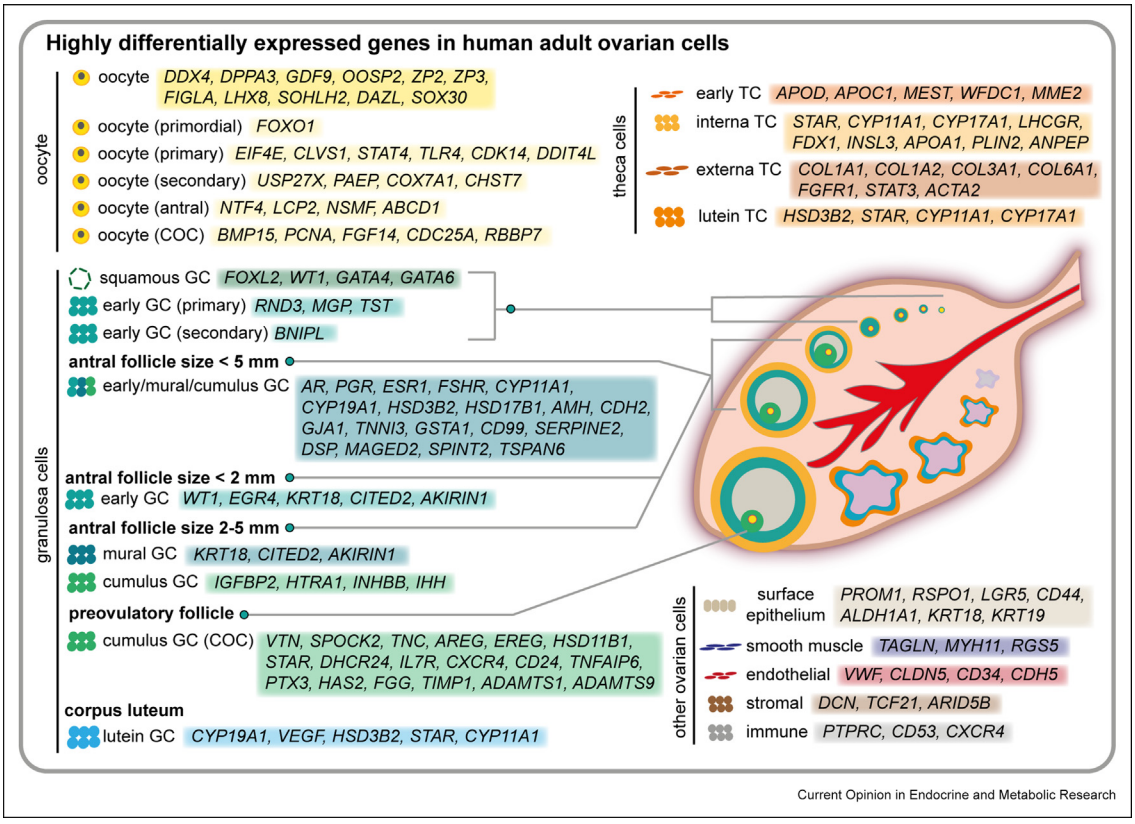
Molecular dynamics of the GC during folliculogenesis

During folliculogenesis, GCs are the only cells that interact directly with oocytes, having a decisive impact on oocyte quality (Figure 2). In primordial follicles (fetal and adult), squamous GCs are known to express *FOXL2*, *GATA6*, *GATA4*, and *WT1* [12,33,34]. Several signaling pathways, such as mTOR, Hippo, and TGFβ superfamily, have been associated with the transition from the primordial (squamous GC) to primary (cuboidal/early GC) follicle [21,35–37]. As per RNA sequencing performed in samples of 10 pooled GCs, GCs from the primary follicle were shown to be enriched in *RND3*, *MGP*, and *TST*; GCs from secondary follicles were enriched in *BNIPL*, whereas levels of several hormone receptors (*AR*, *PGR*, *ESR1*, *FSHR*) and genes related to steroidogenesis (*CYP11A1*, *CYP19A1*, *HSD3B2*, *HSD17B1*) were increased in GCs in antral follicles [13].

Single-cell transcriptomics on 1- to 5-mm antral follicles has revealed that many markers for GCs, such as *AMH*, *CDH2*, *GJA1*, *TNNI3*, *GSTA1*, *CD99*, *SERPINE2*, *DSP*, *MAGED2*, *SPINT2*, and *TSPAN6*, were observed in all GC subtypes [10,11] (See Figure 3). Moreover, the majority of GCs from small antral follicles (1–2 mm) showed high levels of *WT1* and *EGR4*, characteristic of an early GC signature, before differentiation into cumulus and mural GCs. A clear molecular signature separating cumulus and mural GCs was only observed in antral follicles larger than 2 mm, with high levels of *KRT18*, *CITED2*, and *AKIRIN1* observed in mural (and early) GCs and high levels of *IGFBP2*, *HTRA1*, *INHBB*, and *IHH* specifically expressed in cumulus GCs [10] (See Figure 3).

Cumulus GCs collected from cumulus–oocyte complexes from patients undergoing medical assisted reproduction and containing either GV or MII oocytes showed differential expression of cellular matrix components (*VTN*, *SPOCK2*, *TNC*) and genes involved in steroid metabolism and processing (*AREG*, *EREG*, *HSD11B1*,

Figure 3



Highly differentially expressed genes in human adult ovarian cells. Overview of highly differentially expressed genes in main cell types in the ovary (oocytes and somatic cells) in different stages of folliculogenesis. COC, cumulus–oocyte complex; GC, granulosa cell; TC, theca cell.

STAR, *DHCR24*) with upregulation in cumulus GCs associated with MII oocytes [13,38]. Interestingly, immune and inflammation factors are also found upregulated in cumulus GCs associated with MII oocytes [38,39]. In accordance, cumulus GCs isolated at different time points during ovarian stimulation and subsequent ovulation also showed upregulation of inflammation-related genes, such as *IL7R*, *CXCR4*, and *CD24* [40]. Moreover, expression of genes involved in cumulus expansion (*TNFAIP6*, *PTX3*, *HAS2*, *FGG*) and genes potentially involved in follicle rupture and tissue remodeling (*TIMP1*, *ADAMTS1*, *ADAMTS9*) were shown to be increased until ovulation by transcriptomics on cumulus GCs and/or by proteomics of follicular fluid [40,41].

During the formation of the corpus luteum, mural GCs differentiate into lutein GCs (Figures 1b and 2g). In the corpus luteum, CYP19A1 and VEGF are expressed exclusively in lutein GCs, whereas HSD3B2, STAR, and CYP11A1 are expressed by both lutein GCs and lutein TCs [7,42–44] (See Figure 3). During the collection of cumulus–oocyte complexes, additional mural GCs are also aspirated, and those can be transiently cultured and induced to become lutein GCs in vitro. Using this culture model, SCN9A, OTR, and PEDF have been shown to be involved in the regression of lutein GCs [45].

Molecular dynamics of TCs during folliculogenesis

In the ovary, the origin and function of TCs are not well understood, but TCs are known to be involved in diverse aspects of folliculogenesis that include endocrine, structural, vascular, and immune functions [4,46].

Similarly to early GCs in 1- to 2-mm antral follicles, TCs in those follicles show an unique molecular signature that includes expression of *MME2*, *APOD*, *APOC1*, *MEST*, and *WFDC1* [10]. It is only in larger antral follicles that early TCs differentiate into endocrine interna TCs and fibroblast-like externa TCs [47]. In agreement, genes involved in ovarian hormonal biosynthesis and lipid metabolism, such as *STAR*, *CYP11A1*, *CYP17A1*, *LHCGR*, *FDX1*, *INSL3*, *APOA1*, and *PLIN2*, are expressed in the interna TC; by contrast, externa TCs are enriched in genes associated with production of extracellular matrix and vasculature development, such as *COL1A1*, *COL1A2*, *COL3A1*, *COL3A2*, *COL6A1*, *FGFR1*, *STAT3*, and *ACTA2* [10,11,42] (See Figure 3). Interna and externa TCs can be

separated owing to the differential expression of cell surface makers: ANPEP (CD13) is specifically expressed in interna TCs, whereas externa TCs are positive for ENG (CD105) and GPC3 [11]. Lutein TCs sustain the expression of steroidogenic genes, such as CYP17A1 [7,48].

Molecular makeup of the ovarian stroma

Many cell types present in the ovarian stroma can also be found in the stroma of other organs in the body. Those include a variety of immune cells, endothelial cells of lymph and blood vasculature, smooth muscle cells, and fibroblast-like cells. Some of those cell lineages may express organ-specific genes, but they also express canonical lineage markers, such as *PTPRC*, *CD53*, and *CXCR4* in immune cells; *VWF*, *CLDN5*, *CD34*, and *CDH5* in endothelial cells; *TAGLN*, *MYH11*, and *RGS5* in smooth muscle cells; and *DCN*, *TCF21*, and *ARID5B* in fibroblast-like cells [10–12] (See Figure 3). Nevertheless, it is worth noting that each main lineage is heterogeneous, containing several cellular subtypes. For example, the ovary contains several types of immune cells such as monocytes, macrophages, B cells, T cells, and natural killer (NK) cells [10,12], and most probably, those sub types can be further subdivided into additional subtypes. In this regard, also, different subtypes of fibroblast-like cells, perhaps present in different parts of the ovary (tunica albuginea, inner cortex, and medulla), different types of epithelial cells, and smooth muscle cells are responsible for supporting the dynamic structure of the ovary [14]. During the cyclic transition between the follicular and luteal phase, different cell types ensure follicular growth and remodeling, contributing to the great temporal and spatial cellular complexity observed in the ovary.

One of the cell populations that have not yet been captured using single-cell sequencing technologies is the OSE, a single layer of mesothelium cells that covers the ovaries, interfacing with the peritoneal cavity. The role of the OSE is largely obscure. During fetal development, it may contribute to form GCs, whereas in the adult, it may contribute to repair the ovarian surface after ovulation [49]. Regarding this repair function, cells of the OSE express several markers associated with adult stem cells, such as *PROM1* (*CD133*), *RSPO1*, *LGR5*, *CD44*, *ALDH1A1*, and several types of keratins (*KRT18*, *KRT19*), but are negative for *MUC16* (*CA125*) [50] (See Figure 3). The functional relationship between the mesothelium cells of the OSE and the mesothelium cells of the fallopian tubes (fimbria) remains obscure, but they both have been associated with a common type of epithelial ovarian cancer, high-grade serous ovarian carcinoma [51].

Summary

The transcriptional signature of oocytes, GCs, and TCs among all other cells present in the adult ovary is starting to reveal the cellular complexity and dynamics of the human ovary. In future, including more samples of different phases of the ovarian cycle and perhaps ovarian tissue from patients with different ages and reproductive diseases will allow a more complete understanding of the broad range of cell types in the ovary, which will benefit the development of disease models. Moreover, the differentiation in vitro of human competent oocytes, starting from primordial follicles, is still a challenge. To mimic follicle development in vitro, the molecular analysis of ovarian cells, including oocytes, GCs, and TCs, during culture will allow efficient optimization of culture protocols to achieve oocyte maturation, providing innovative solutions for medical assisted reproduction and fertility preservation.

Conflict of interest statement

Nothing declared.

Acknowledgements

The authors would like to acknowledge J.S. Del Valle and E.M. Lam for the histological images (hematoxylin and eosin staining) of human adult ovaries used in Figure 2. This work was supported by the European Research Council Consolidator Grant OVOGROWTH (ERC-CoG-2016-725722) and the Chinese Scholarship Council (CSC 201706320328).

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This article described somatic cell types and transcriptional signatures of human adult ovarian cells from medulla and antral follicles sized 1–5 mm by single-cell RNA sequencing. Several subtypes of endothelial, smooth muscle and immune cells were confirmed in human adult ovary. The analysis also revealed features of early GC and early

TC. Moreover, this paper also identified novel markers for GC and TC in antral follicles.

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This article described the transcriptional profiles of GC and TC from antral follicles (1–4 mm) by single-cell RNA sequencing and included mouse-xenografted ovarian tissue. In addition, this paper applied several cell surface markers to sort GC and TC and validated some of those on tissue sections.

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