

POSTER VIEWING
STEM CELLS

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P-786 Mesenchymal stem cells as a potential source of ovarian theca interna cell differentiation

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Study question: Are mesenchymal stem cells (MSCs) the cell source of origin during the differentiation of ovarian stromal cells into theca interna cells (TICs)?

Summary answer: MSC-like cells were observed during *in vitro* TIC differentiation, particularly on day 2, suggesting their potential role in the origin of TICs.

What is known already: Folliculogenesis is the process during which a follicle develops from its primordial towards its antral stage. Follicles in the antral stage consist of an oocyte, granulosa and theca cells. While the oocyte is surrounded by granulosa cells in the primordial stage, theca cells differentiate from their precursor cell from the secondary stage onwards. Recent studies identified the existence of theca stem cells (TSCs) in both humans and animals. These TSCs express both platelet-derived growth factor receptor (PDGFR) and nerve growth factor receptor (NGFR), and demonstrate the ability to differentiate into mesenchymal cell lineages, resembling characteristics of MSCs.

Study design, size, duration: Ovarian stromal cells were isolated from cryopreserved cortex fragments, and cultured *in vitro* for 13 days, using cell

media enriched with various growth factors and hormones, as previously established by our group. Flow cytometry, immunocytochemistry, reverse transcription-quantitative polymerase chain reaction (RT-qPCR), and enzyme-linked immunosorbent assay (ELISA) were employed to analyze the cells and their media at days 0, 2, 8 and 13.

Participants/materials, setting, methods: Whole ovaries from three postmenopausal organ donors were obtained (50, 54, and 64 years) and the cortex was cryopreserved in small 5x5 fragments prior to the isolation and differentiation into TICs.

Main results and the role of chance: Immunocytochemistry results showed an increase in the proportion of cells positive for TIC markers from day 0 to day 8 ($12.13 \pm 2.11\%$ vs. $57.73 \pm 49.99\%$), while the proportion of MSC-like cell markers was increased significantly from day 0 to day 2 ($4.54 \pm 1.71\%$ vs. $57.34 \pm 18.05\%$). This TIC proportion aligns with the rising androstenedione levels from day 2 to day 13 (81.31 ± 25.09 pg/ml vs. 384.29 ± 245.82 pg/ml). Flow cytometry analysis identified a $CD73^+ CD90^+ CD105^+ CD34^- CD45^- HLA-DR^-$ cell population, expanding from less than 1% on day 0, to $15.30 \pm 7.9\%$ on day 2. A $CD13^+$ cell population and its corresponding gene expression increased significantly from the beginning towards the end of the culture ($13.52 \pm 7.9\%$ vs. 84.13 ± 11.08 , and 1.38 ± 0.97 vs. 285.80 ± 27.43 , flow cytometry and gene expression analysis respectively). Moreover, NGFR and PDGFRA expression was induced on day 0 and day 2, respectively, compared to day 13 (1.12 ± 0.60 vs. 0.08 ± 0.08 , and 2.03 ± 1.11 vs. 0.24 ± 0.14). In conclusion, our study provides valuable insights into the differentiation potential of TICs, suggesting a possible origin from MSCs.

Limitations, reasons for caution: Due to the limited accessibility of a large quantity of human ovarian tissue from postmenopausal women, this study is restricted to three biological replicates. When examining statistical differences, it is crucial to bear in mind the considerable biological variabilities that exist among humans.

Wider implications of the findings: Characterizing the origin of TICs will allow for a comprehensive study of TICs, leading to a broader knowledge of these cells. The *in vitro* differentiation to TICs from easily accessible cells, like MSCs, holds potential significance in toxicity studies and investigating TIC-related disorders, such as polycystic ovary syndrome (PCOS).

Trial registration number: not applicable



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