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Heterogeneous cell population derived from human ovarian follicular liquid: morphological studies and molecular screening

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The origin of oocytes and primary follicles in ovaries of adult mammalian females is still a matter of dispute [1]. The components of new primary follicles, primitive granulosa and germ cells, differentiate sequentially and de novo from mesenchymal progenitor cells residing in the ovarian tunica albuginea (TA). It appears that mesenchymal progenitor cells contribute to the generation of epithelial cells similar to granulosa cells (GCs). The multipotency of a subset of granulosa cells was also established by in vitro differentiation into other cell types [2]. Up to now, luteinizing GCs were considered to be terminally differentiated, unavoidably becoming apoptotic a few days after ovulation. Previously, we have provided evidence for the existence of putative stem cells derived from human ovarian follicular liquid collected after routine procedures for in vitro fertilization techniques [3]. These cells grow in minimal medium condition, without any growth factor (i.e. LIF), that is considered essential according to other procedures [4]. Using immunocytochemistry and flow cytometry we showed that these cells are positive for several mesenchymal stemness markers, including CD90, CD73, CD44, CD105. However, morphological analysis revealed a heterogeneous cell population, with cells displaying a fibroblast-like, epithelial-like and neural-like shapes. These observations are also supported by the identification of cells expressing specific neural markers, such as neurofilaments and PGP9.5, in addition to vimentin and cytocheratin positive cells. All these data are suggestive of the presence of different cell populations in follicular fluids. To verify this hypothesis we select a panel of markers specific for the different cell populations previously identified and we plan a molecular screening to follow their expression in the follicular fluid derived cells at different times of minimal culture conditions in vitro. Bone marrow derived MSCs were used as a control. For each sample we performed semiquantitative RT-PCR experiments normalizing the cDNAs used as templates on the basis of the number of pseudo-mesenchymal cells morphologically identified in the sample. For this purpose OCT-4 was selected as a stem marker to follow the mesenchymal stem cell population, while FSH-R was used to identify granulosa derived cells; CNTF and beta-3-tubuline were used to discriminate between neural and neuronal cells populations; epithelial and hematopoietic cells were followed using cytokeratin (CK8 and CK10) and CD45 markers, respectively. GAPDH and β-actin specific primers were used on all samples for normalization. Here we compare the results of this molecular screening with the previously obtained immunocytochemical and morphological data to confirm the presence of these different cytotypes in the samples purified from the follicular liquid and their persistence, loss or amplification at different times of in vitro minimal culture conditions.

References

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