The role of oocyte-secreted factors in the human granulosa cell line - GC1a

SA Reis[1], A Ribeiro[1,2], JL Silva-Carvalho[1,2,3], AM Gouveia[1,4], H Almeida[1,3]

[1] Departamento de Biologia Experimental, Faculdade de Medicina, IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto.
[2] CETI – Centro de Estudo e Tratamento da Infertilidade, Porto.
[3] Hospital CUF, Porto.
[4] Faculdade de Ciências da Nutrição e Alimentação, Universidade Do Porto.

The success of assisted reproductive techniques (ART) is partly dictated by the oocyte quality, achieved during its development. A major contribution for oocyte quality is provided by the follicular micro environment that includes the follicular fluid and the oocyte adjacent cells. These comprise granulosa cells (GCs) that multiply along oocyte growth and development and differentiate into two structurally and functionally distinct types: the mural GCs and cumulus cells (CCs). The oocyte is an indirect regulator of itself since it can modulate follicular cells function. In fact, it produces the oocyte-secreted factors (OSFs), which are TGFβ superfamily growth factors that promote follicular cells survival that, in turn, modulate oocyte growth.

In order to increase our understanding on the role of OSFs in GCs, and also aiming to uncover molecules with potential properties of clinical biomarkers, we employed an immortalized human granulosa cell line, the GC1a type. This appears to circumvent considerably the lesser progress attained when primary culture of human granulosa cells are employed instead.

In this work, we aimed to characterize the dynamic process between GCs and OSFs, in GC1a cells. To investigate the presence of OSFs and their receptors in GC1a, the total mRNA was extracted and converted to cDNA to analyze gene expression by real-time PCR. To study the effects of OSFs, present in the follicular fluid, GC1a cells were incubated with different concentrations of this fluid. Firstly cells viability was determinated by MTT assay and the study of cell morphology was established by microscopic analysis, assessed by actin staining.

The experiments made so far evidenced OSFs transcripts, namely GDF7, GDF9, and also the OSFs receptors, such as BMPRI, BMPRII, TGF β RI, TGF β RII, ACVR II, ALK3, reported for the first time in this cell line. Concerning the effects of follicular fluid in cell culture, the results suggest that concentrations higher than 50% of follicular fluid in the cells medium interfere with cell viability. Additionally, the follicular fluid seems to interfere with cellular morphology, since cells lose their epithelial like morphology and acquire a more closed fibroblastic shape, with cellular protrusions, suggesting that follicular fluid may induce cytoskeleton reorganization.

The data suggest that this cell line mimics CCs cells, regarding the presence of OSFs and their, and may thus be employed in the study of OSFs signaling. As a whole, the study establishes a consistent ground for future work in the TGF β transductive pathways.