Emerging Roles of Filamin A in the Human Ovary and Granulosa Cells

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Filamin A (FLNA) was identified as a cytoskeleton associated protein that crosslinks actin filaments. By now, it is clear that it is a multifunctional protein, which serves as an intracellular signaling scaffold, coordinating pathways linked to cell differentiation, development, and morphogenesis. FLNA is widely expressed in various human cell types and roles in certain tumors have been discussed in recent years. Yet, surprisingly FLNA and its roles have not been addressed in the human reproductive system, in which morphogenic events and cell differentiation are of great importance. We recently initiated such studies. In the human male gonad, we described FLNA in germ cells and peritubular cells, and shed light on its role in seminoma. We now address the role of FLNA in the female gonad. Data mining, including transcriptomic and proteomic data, suggested the presence of FLNA in various human ovarian cell types, including granulosa cells and oocytes. Immunohistochemical examinations of sections from human and nonhuman primate ovaries demonstrated robust expression of FLNA in follicular granulosa cells, luteinized granulosa cells in the *corpus luteum,* as well as oocytes. Further investigations using fluorescence microscopy and Western blotting (WB) uncovered the sustained expression of FLNA in cellular models, specifically in cultured *in vitro* fertilization-derived granulosa cells (IVF-GCs) and the human granulosa-like tumor cell line KGN. Moreover, our observations indicated a downregulation of FLNA in response to forskolin and/or hCG treatment in both cell types (IVF-GCs: qPCR n = 13, WB n = 8; KGNs: qPCR n = 6, WB n = 6; statistical significance determined by Student's t-test $\alpha = 0.05$). To gain insights into the functions of FLNA in the human ovary, we conducted immunoprecipitation experiments followed

by mass spectrometry analysis in IVF-GCs (n = 4) and KGN cells (n = 3). Among the top 20 proteins identified for each cell type, there was an overlap of 8 interaction partners, including stress-70 protein (HSPA9) amongst others, in addition to cell-type specific interactors. Notably, in GCs proteins with important cell-specific roles, namely the enzyme cholesterol side-chain cleavage (CYP11A1), stress-70 protein (HSPA9) and gap junction alpha-1 (GJA1) were identified. Currently, further studies are under way to examine their intracellular co-localization. In summary, our results reveal the presence of FLNA in the ovary, in IVF-derived GCs as well as KGNs, its regulation in response to FSK/hCG, and its interaction partners with physiological significance relating to steroidogenesis, mitochondrial import and cell-cell communication. *(Supported by DFG project number 491030536)*.