IS–55 Na⁺/K⁺-ATPase Activity in Porcine Granulosa Cells is Closely Related to the Cell Proliferation and Apoptosis

Department of Obstetrics and Gynecology, Kobe University School of Medicine, Japan
Ahmed ALJONAIID, Asomi SATO, Saiko ASAHARA, Takeshi MARUO

[Objective] To investigate the biological role of Na⁺/K⁺-ATPase in the regulation of proliferation and apoptosis of granulosa cells during follicular growth, Na⁺/K⁺-ATPase activity and cell proliferation/apoptosis in porcine granulosa cells were studied in the presence or absence of epidermal growth factor (EGF) which has been shown to have stage-specific diverse effects on granulosa cell functions. [Methods] Porcine granulosa cells collected from small (1–2 mm) or large (6–11 mm) follicles were cultured in a serum-free condition with or without EGF (100 ng/ml) for 48 h. The activity of Na⁺/K⁺-ATPase was measured by Esmann's method. Apoptosis was assessed by TUNEL method. [Results] Na⁺/K⁺-ATPase activity was markedly prominent in granulosa cells from small follicle relative to that from large follicle. The increased activity of Na⁺/K⁺-ATPase in granulosa cells corresponded to the proliferative activity based on PCNA expression in the cells. Large follicle granulosa cells had decreased Na⁺/K⁺-ATPase activity. Treatment with EGF augmented the enzymatic activity of Na⁺/K⁺-ATPase and decreased apoptosis in small follicle granulosa cells, but decreased Na⁺/K⁺-ATPase activity in large follicle granulosa cells. [Conclusion] Na⁺/K⁺-ATPase may have a vital role in regulating the proliferation and apoptosis of porcine granulosa cells during follicular growth.


Department of Obstetrics and Gynecology, Asahikawa Medical College, Japan
Bochen PAN, Kazuo SENGOKU, Naoyuki TAKUMA, Keiko TSUCHIYA, Harumi KOMORI, Yukio WATANABE, Mutsuo ISHIKAWA

[Objective] To investigate the expression and function of heparin-binding epidermal growth factor-like growth factor (HB-EGF), a relatively new member of the EGF growth factor family, in human luteinized granulosa cells (LC). [Methods] Human LC obtained from patients undergoing IVF treatment were cultured in serum-free or low serum culture medium and were treated with either human recombinant HB-EGF or CRM197, a specific inhibitor for interaction of HB-EGF with the cognate receptors. Apoptosis of LC was studied with hematoxylin staining of the nuclei and was confirmed by TdT-mediated dUTP-biotin nick end labeling (TUNEL). Detection of HB-EGF protein was done with Immunocytochemistry. Relative mRNA expression levels of HB-EGF and the EGF receptor family were measured with semiquantitative RT-PCR. Informed consent was obtained from all patients. [Results] 1) Both mRNA and protein of HB-EGF were expressed in the cultured LC. 2) CRM197 added to the culture medium caused profound apoptosis of LC. 3) Addition of human recombinant HB-EGF added to the culture markedly inhibited the apoptosis induced by serum deprivation. 4) Human recombinant HB-EGF stimulated acute expression of HB-EGF mRNA. 5) All the four members of the EGF receptor family were expressed in the LC, but the level of ErbB4 mRNA was very low. [Conclusion] HB-EGF may play essential roles in supporting the human corpus luteum in an autocrine or paracrine fashion.

IS–57 Somatic Cells within Abnormal Follicles of the Female ArKO Mouse Exhibit Characteristics of Adult Sertoli Cells

Prince Henry's Institute of Medical Research, Clayton 3168, Victoria, Australia
Kara Brit, Ann Drummond, Liza O'Donnell, Margret Jones, Jock Findlay and Evan Simpson

Female aromatase knockout (ArKO) mice, generated by targeted disruption of the Cyp19 gene, are infertile, with folliculogenesis arrested at the antral stage, and no corpora lutea. The ovaries of 22–week-old mice maintained on a soy-free diet display both healthy and atretic follicles. The ovaries also exhibit numerous abnormal follicles within diffuse interstitium and haemorrhagic cysts. Light microscopy demonstrated that the somatic cells within these abnormal follicles displayed adult-type Sertoli cell characteristics, such as a basally located nucleus containing a defined nucleolus, and an extensive cytoplasm with lateral, cylindrical-like processes. Electron microscopy demonstrated the homogeneous distribution of chromatin within the nucleus, and the presence of specialised junctions between adjacent cells. Desmosome-like adherens junctions were observed, closely resembling gap junctions seen in normal follicles. Importantly, ectoplasmic specialisations, which are highly specialised junctions specific to Sertoli cells in the testis, were observed. Immunocolocalisation of an ectoplasmic specialisation-specific protein established that this protein was present within the Sertoli-like cells of the abnormal follicles, but in normal follicles within either the ArKO or wildtype ovary. Real time PCR analysis of SOX-9 showed that wildtype ovaries express SOX-9, and thus the expression of this gene could not be used as a marker of Sertoli cells. We conclude that ArKO ovaries display numerous follicles containing somatic cells characteristic of adult-type Sertoli cells. Given that these cells are not prominent in younger animals, we speculate that in the absence of estrogen, granulosa cells may differentiate into Sertoli cells.