Novel function of ovarian growth factors: combined studies by DNA microarray, biochemical and physiological approaches

Abraham Amsterdam

Ada Dantes

Sarit Freimann

Ido Ben-Ami

Leah Armon

Gonadotropin hormones control the main functions of the human ovary. Luteinizing hormone (LH) surge, which occurs prior to ovulation, triggers a cascade of events in the ovarian follicle including resumption of meiotic maturation; differentiation of mural granulosa cells, usually referred to as luteinization, reprogramming of their protein and steroidogenic activity; expansion or maturation of the cumulus cells surrounding the oocytes; and, finally, with rupture of the follicle wall and release of a fertilizable ovum.

Although intensive and extensive research has been conducted in order to decipher the main factors that cause oocyte maturation and ovarian follicle rupture, the genes modulated in response to gonadotropins stimulus in isolated human granulosa cells, obtained from women undergoing in vitro fertilization (IVF) treatment has only recently been described by us.

Studying amphiregulin and epiregulin as LH mediators

Ar and Ep require cumulus cells to exert their effects, since denuded immature oocytes do not undergo oocyte maturation. According to the classic paradigm, cAMP, generated by the cumulus cells, is transmitted to the oocyte via gap junctions, and serves as «meiotic arrestor». It was hypothesized that LH surge induces resumption of meiosis by the breakdown of these gap junctions, which terminates the supply of cAMP from the cumulus cells to the oocyte. However, since the restricted expression of LH receptor on both the cumulus cells and the oocyte itself, many LH effects are thought to be indirect. Recently, it was found both in rodent and human that amphiregulin (Ar) and epiregulin (Ep) of the epidermal growth factor family were dramatically up regulated by gonadotropins in the intact ovary and in primary granulosa cells respectively (Table I). Their role in cumulus expansion and oocyte maturation was established in rodent, and their formation under LH stimulation in granulosa cells was demonstrated in humans. It is therefore hypothesized that Ar and Ep may act downstream of LH in an indirect, paracrine, cumulus depended manner (Figure I).

Studying LH induced amphiregulin and epiregulin signaling pathway

Recently, it was demonstrated that LH induced biosynthesis of Ar and Ep in primary human granulosa cell is mediated at least in part by c-AMP/PKA cascade. Ar and Ep are synthesized as integral membrane precursors with a single transmembrane domain. In order to gain biological activity they must undergo a specific proteolytic cleavage of the ectodomain at the membrane surface by members of disintegrin and metalloproteinases (ADAMs). Interestingly, this shedding process was found to be regulated by GPCR signaling. It was demonstrated that a wide range inhibitor of ADAMs reduces the activation of EGF receptor in rat follicles, thus interfering ovulation process. However, which of the ADAMs family member is involved in Ar and Ep processing is yet to be determined.

Studying the involvement of amphiregulin and epiregulin in carcinogenesis

Both Ar and Ep were reported to be involved in various cancers. Ar and its receptor, EGFR, were expressed in a series of invasive ductal breast carcinoma specimen. Ep was found to be involved in the stimulation of tyrosine phosphorylation of ErbB-4 and EGFR in human breast carcinoma cell lines. Ep exerts a mitotic activity in various primary cell types such as rat hepatocytes, as well as in various types of human cancer cell lines, especially in epithelial tumor cell lines. Recently, an involvement of Ep expression in tumorigenesis through activated Ki-RAS signaling pathway was suggested in human colon cancer cells. Likewise, Ep was found to be upregulated and stimulated growth of human pancreatic cancer cells. Furthermore, it was demonstrated that Ar might be involved in the pathogenesis and outcome of human ovarian cancer.

Department of Molecular Cell Biology

972 8 934 3713

972 8 934 4125

Abraham.Amsterdam@weizmann.ac.il

www.weizmann.ac.il/mcb

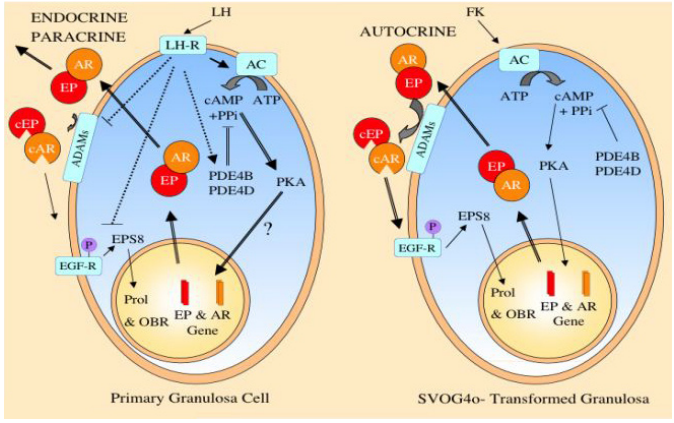

Fig 1. *Tentative role of LH stimulation of amphiregulin and epiregulin biosynthesis and control of ovulation in mammalian follicle. Figure1: LH binds to its receptor on granulosa cell (LH-R), activates adenylate cyclase (AC), which elevates intracellular c-AMP, thus stimulates protein kinase A (PKA), which leads to de novo formation of amphiregulin (Ar) and epiregulin (Ep). Ar and Ep are specifically cleaved by metaloproteinases (ADAM) associated with the cell membrane. These activated growth factors can either bind and activate EGFR and ErbB4 on the adjacent cumulus cells (paracrine loop), thus triggering cumulus expansion and oocyte maturation, or bind to the same cell (autocrine loop), thus exerting mitogenic effect. In normal granulosa cells, following LH stimulation there is downregulation of ADAMs and EPS8, which may attenuate the autocrine loop. However, this down regulation is not evident transformed granulosa cells (SVOG4o).*

			Fold change above control		
Gene	**Accession No.**	**Abbreviation**	**LH**	**FSH**	**FK**
amphiregulin (schwannoma-derived growth factor)	NM_001657.1	AREG	286.2	41.8	859.0
epiregulin	NM_001432.1	EREG	60.3	14.9	66.7
insulin-like growth factor 2 (somatomedin A)	X07868	IGF2	41.8	25.8	73.0
insulin-like growth factor 2 receptor	BG031974	IGF2R	32.5	41.8	#-1.2
insulin-like growth factor-binding protein 4	M62403	IGFBP4	29.3	17.4	5231.0
transforming growth factor-beta type III receptor	L07594	TGFBIIIR	27.1	3.6	#1.3
vascular endothelial growth factor	AF091352.1	VEGF	32.0	14.4	4.8
ryanodine receptor 3	AJ001515	RYR3	27.9	26.7	14.5
luteinizing hormone/choriogonadotropin receptor	M63108	LHCGR	16.9	20.7	14.0
transducer of ERBB2, 1	AA675892	TOB1	7.4	2.3	2.3
PDGFA associated protein 1	U41745	PDAP1	5.7	5.7	#-1.8
growth arrest and DNA-damage-inducible, gamma	NM_006705.2	GADD45G	4.6	11.7	2.2
endothelial differentiation-related factor 1	AB002282.1	EDF1	3.4	4.6	#1.4

Table 1: *Upregulated genes in primary human granulosa cells as detected by DNA microarray.*

Data are the mean of four determinations. Cells were challenged with 3IU/ml of hLH or 3IU/ml of hFSH or 50mM of forskolin (FK) for 24h at 37°C. # changes are not statistically significant p>0.05.

Once Ep and Ar , which are known to be mitogenic, are generated and secreted from the granulosa cells, they might exert their effect not only on the cumulus cells as paracrine factors, but also on the same membrana granulosa cells that produce them in an autocrine loop. Since Ar and Ep are preferentially paracrine, cumulus dependent mediators, it was hypothesized that following LH surge, down-regulation mechanism exists, which attenuates the autocrine loop, thus preventing these growth factors potential to act as mitogens on the granulosa cells themselves. Indeed, our data from DNA microarray studies showed a significant reduction in gene activity of ADAMTS1 and ADAM12 following LH stimulation. Furthermore, though there was no significant change in either ErbB-4 or EGFR expression, a significant downregulation of epidermal growth factor receptor substrate 8 (EPS8), which is essential for the mitogenic signals from the phosphorylated EGFR was demonstrated. Finally, based on RT-PCR technique, our recent data confirmed that ADAMTS1 and ADAM12 mRNA levels are downregulated following LH stimulation of primary human granulosa cells. Interestingly, in contrast to this downregulation, which was concomitant with upregulation of Ep and Ar expression, no change in ADAMTS1 and ADAM12 mRNA levels were observed in SV-40 transformed human granulosa cells. This data suggests that loss of this downregulation may be involved in the development of ovarian tumors.

Selected publications

Rimon, E., Sasson, R., Dantes, A., Land-Bracha, A., and Amsterdam, A. (2004) Gonadotropin-induced gene regulation in human granulosa cells obtained from IVF patients: modulation of genes coding for growth factors and their receptors and genes involved in cancer and other diseases. Int J Oncol 24, 1325-1338.

Sasson, R., Rimon, E., Dantes, A., Cohen, T., Shinder, V., Land-Bracha, A., and Amsterdam, A. (2004) Gonadotrophin-induced gene regulation in human granulosa cells obtained from IVF patients. Modulation of steroidogenic genes, cytoskeletal genes and genes coding for apoptotic signalling and protein kinases. Mol Hum Reprod 10,299-311.

Freimann, S., Ben-Ami, I., Dantes, A., Ron-El, R., and Amsterdam, A. (2004) EGF-like factor epiregulin and amphiregulin expression is regulated by gonadotropins/cAMP in human ovarian follicular cells. Biochem Biophys Res Commun 324, 829-834.

Freimann, S., Ben-Ami, I., Dantes, A., Armon, L., Ben Ya'cov-Klein, A., Ron-El, R., and Amsterdam, A. (2005) Differential expression of genes coding for EGF-like factors and ADAMTS1 following gonadotropin stimulation in normal and transformed human granulosa cells. Biochem Biophys Res Commun 333, 935-943.

Ben-Ami, I., Freimann, S., Armon, L., Dantes, A., Ron-El, R., and Amsterdam, A. (2006) Novel function of ovarian growth factors: combined studies by DNA microarray, biochemical and physiological approaches. Submitted for publication. Mol Hum Reprod.

Acknowledgements

A. Amsterdam is the incumbent of the Joyce and Ben B. Eisenberg Professorial Chair in Molecular Endocrinology and Cancer Research at the Weizmann Institute of Science.

This study was supported by Yad-Avraham Center for Cancer Research, by a research grant from Mr. Edgar L. Cadden and La Fundation Raphael et Regina Levy, and by the Women Health Research Center at the Weizmann Institute of Science, Rehovot, Israel.