Mechanisms of Apoptosis in the Ovary

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Abstract
Of ovarian follicular reserve, only a small part of these follicles reach maturity, most of them suffering an involution process called follicular atresia. In addition, for the initiation of a new wave of follicular development, a prerequisite is the corpus luteum regression. Last year studies showed that the two processes taking place in the ovaries are based on a complex mechanism, of genetically programmed cell death, significantly called apoptosis, a process conserved in terms of evolution, by which cells inactivate, disassemble and degrade their own structural and functional components systematically for completion of their own death. Ability of cells to enter in apoptosis in response to a specific death signal, depends on both their proliferative status and position in cell cycle, and on controlled expression of genes that promote, inhibit or affect cell death program. Any dysfunction that occurs in any of the key points of the programmed death can lead to erroneous apoptotic process, resulting in expression of pathological conditions.

Keywords: apoptosis, death receptor, mechanisms, mitochondrial pathway, ovary

1. Introduction

Two mechanisms by which a cell "suicide" by apoptosis were studied in the ovary, a mechanism generated by the signals occurring within the cell and which involves mitochondria and protein family members BCL-2 (mitochondrial of apoptosis) and another one triggered by death activators that bind to complementary receptors on the surface of target cells (death receptor).

1. Ovarian apoptosis triggered by internal signals (mitochondrial pathway)

Active apoptosis occurs in the ovary, in germ cells and also in granulosa cells, internal sheath cells and in the luteal cells, cells where mitochondria and BCL-2 family members have an essential role. Among these, anti-apoptotic members (BCL-2 and BCL-xL), which are located on the external mitochondrial membrane, support survival of the cell by maintaining mitochondrial membrane integrity.

In normal physiological situations, BCL-2 protein is coupled to a protein molecule, Apaf-1 (Factor 1 of apoptotic proteases activation), thereby preventing leakage of mitochondrial proteins in the cell cytoplasm. Endogenous emergence of negative signals (e.g. insufficient hormonal stimulation or occurence of ROS) or internal damage to the cell, causes the release of protein Apaf-1 and activation of pro-apoptotic proteins type Bax. Bax fraction, by homodimerisation disrupts mitochondrial membrane permeability and transient appearance of a pore at their level, which causes the release of mitochondrial proteins, two of them aving an important role in apoptosis: cytochrome c and the apoptosis inducing factor (AIF) [1-4].

The release of cytochrome c induces release of caspases, especially of procaspase 9, protein enzyme responsible for cell death. In order for this process to take place, cytochrome c must first bind to the Apaf-1 protein released, causing its conformational change. In addition, Apaf-1 molecule presents a connection metabolic site for

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d'ATP, a cofactor of apoptosis. Multiproteic complex consisting of cytochrome c, molecule Apaf-1 and d'ATP is called apoptosome complex, which then binds and activates procaspase 9, thereby initiating a caspase cascade (Figure 1) [5-9].

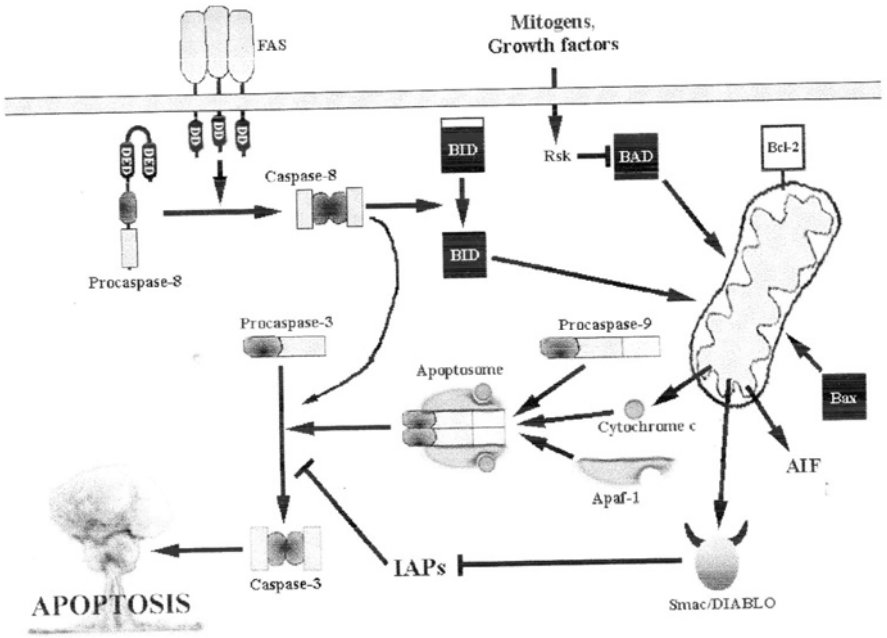

Figure 1. Mitochondrial pathway of apoptosis. Enabling of apoptosome complex (after Vaskivuo, T., 2002)

2. Ovarian apoptosis triggered by external signals: the of death receptor

Death receptors are transmembrane proteins that belong to the super-family of Tumor necrosis factor (TNF - Tumor Necrosis Factor), with the death receptor exposed on the cell surface for attaching additional ligand. Of the 20 existing receptors, responsible for apoptotic signaling in the different cell types, two are found mainly in the ovaries, namely TNFR1 (Tumor Necrosis Factor Receptor 1; DR1) and Fas receptor (CD 91, DR2, Apo1) [2, 8, 10, 11].

Death receptors can activate and trigger the caspases cascade within seconds after ligand attachment, induction of apoptosis by this mechanism being very fast. Ligand- receptor complex formation causes the triggering of growth and/or decrease of the intracellular concentration of second order messengers, eg.: cAMP, Ca^{2+}, ceramide, 1,2-diacilglicerol, mediating brief cellular responses.

2.1. Cell death signaling through Fas–FasL system

Fas receptor is a glycoprotein of 45 kDa possessing two domains, death domain (DD - Death Domain) and death effector domain (DED - Effector Death Domain). Coupling Fas ligand (FasL) with Fas receptor causes conformational changes through trimerisation and intracellular grouping of death domain (DD). Receptor activation allows selection and attachment to it of some adapter molecules, FADD type (Fas Associated Death Domain) via homologous death domains (adapter molecules contain both the DD and DED) and the onset of apoptosis by recruiting and activating procaspase 8 (Figure 2) [11-13].

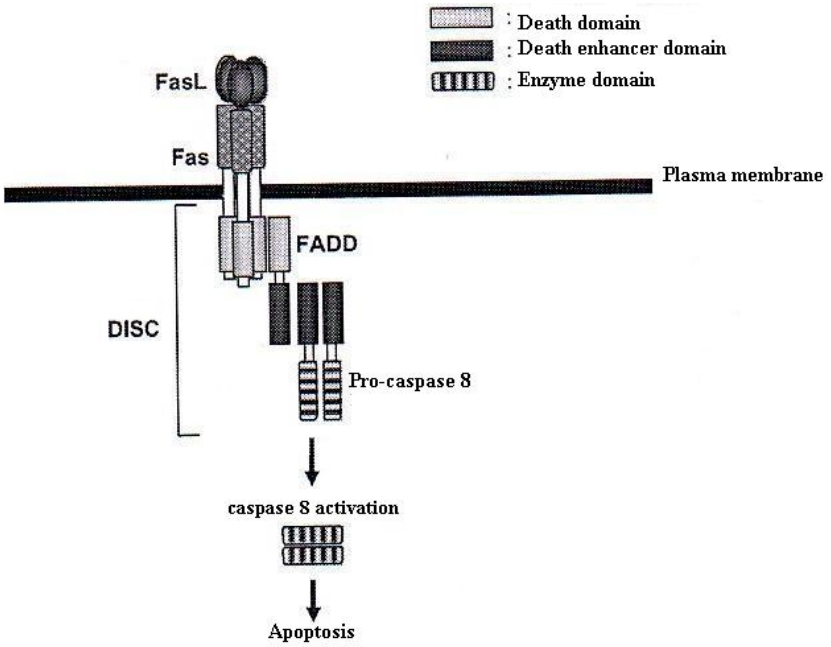

Figure 2. Signaling of of cell death through receptor Fas (CD 95 / Apo-1)
(after Matsuda-Minehata, F. et al., 2008)

Multiproteic FasL-Fas complex - adapter molecule - initiator procaspase 8 was called DISC complex (Death Inducing Signal Complex).

Lévy, [2] found that in mice Fas receptor is present on the surface of primordial follicle oocytes, primary and secondary, and interaction of Fas-FasL triggers apoptosis in these cells. The pro-apoptotic effect of Fas-FasL system was observed in granulosa cells frequently, in several species: in rodents [14], women [15], in pigs [16] and cattle [17, 18]. In addition, Fas - FasL system involvement granulosa cells apoptosis was also demonstrated by "in vitro" experiments. Stimulation of Fas signaling can induce apoptosis in granulosa cells from primary culture, in several species, eg. mice, rat, cattle and human [17, 19, 20].

Regarding the involvement of Fas-FasL complex in luteal cell apoptosis, numerous studies [17, 19, 21, 22] concluded that both receptor Fas and FasL are present in luteal cells, the level of both of them being high during luteolisis. However, in rat, system Fas-FasL mediates apoptosis induced by prolactin in luteal cells.

2.2. Cell death signaling through TNFα-TNFR1 system

TNFα is a polypeptide of 17 kDa, with a dual function, of cell death ligand but also of survival and proliferation factor.

Attaching the ligand TNFα to receptor TNFR1, holder of a DD domain, stimulates apoptotic signaling path and interaction of TNFα with TNFR2 determines cell survival.

On apoptotic path, interaction of TNFα with TNFR1 receptor determines trimerisation receptor and intracellular clustering of death domain (DD), which allows the attaching of a TRAAD adapter molecule (TNFR Associated Death Domain), through the interaction between DD domains. TRADD is able to recruit a number of proteins to the activated receptor. Thus, the association between TRAAD and FADD (Fas Associated Death Domain), result in triggering apoptosis by recruiting and activating procaspase 8 [11, 23, 24].

Studies by Lévy, [2] have emphasized the involvement of TNFα-TNFR1 signaling path in apoptosis in rat oocytes. Also Praj - Kiel, [25] found that in swine granulosa cells both TNFR1 and TNFR2 express, and TNFα can induce proliferation or death of these cells, thus a balance being needed between pro and antiapoptotic factors. In addition, Nakayama,

[26] found that in pig ovaries, the level of TNFα in granulosa cells increases during atresia and TNFR2 is strongly expressed in granulosa cells coming from healthy follicles and decreased during follicular involution.
In pigs, Pate, et al., [27] concluded that both synthesis and action of oestradiol, which in this species has luteotrophic role, are inhibated by TNF-α. In normal physiological situations, luteal cells in pigs, under the influence of PGF2α, synthesize high amounts of progesterone. Under the action of TNFα, PGF2α cause a reduction of the progesterone level by transforming luteotrophic action of PGF2α in luteolitic action by the local release of cytokines. They mediate increase of fosfolipase level, the appearance of reactive oxygen species and, ultimately, triggering of the apoptosis.
TNFα receptors were located both in the large luteal cells, but especially in the small luteal cells, which apparently have a particular affinity for them. In addition, Friedman, et al., [28] found that endothelial cells in the corpus luteum also have receptors for TNFα.
In cattle, TNFα alone does not affect luteal cell viability, but in the presence of IFNγ it exerts a cytotoxic effect. If TNFα and its receptor are present on the surface of luteal cells, IFNγ directly induce apoptosis of luteal cells by mediating the luteolitic action of PGF2α and appearance of ROS. Although ROS do not initiate luteal regression, they are responsible for the events that form up the luteal regression cascade and that end with corpus luteum involution [27].

2. Conclusions

Ovarian apoptosis is actively manifested both in germ cells and in granulosa cells, internal sheath cells, and also in the luteal cells.
There are two mechanisms in the ovaries by which a cell "suicide" through apoptosis, namely the mechanism of mitochondrial path and that of death receptor.
The mitochondrial way of apoptosis concerns members of protein family Bcl-2, with disruption of mitochondrial membrane permeability and the appearance of a transient pore, release of mitochondrial proteins, two with important role in apoptosis: cytochrome c and the apoptosis inducing factor (AIF).
The death receptor way involves members of tumor necrosis factor family (TNFR1 and Fas receptors), which activate and triggers cell death cascade within seconds of the complementary ligand attachment.

References

1. Svanberg, B, Apoptosis- the cellular mechanism of rat ovarian follicular atresia, Department of Physiology, Göteborg University, Sweden, 2000.
2. Lévy, R., Apoptosis in oocyte, Gynécology Obstétrique-Fertilité, 2005, 33 (9), 645-652.
3. Gürsoy, E., Ergin, K., Başaloğlu, H., Koca, Y., Seyrec, K, Expression and localisation of Bcl-2 and Bax proteins in developing rat ovary, Research in Veterinary Science, 2008, 84, 56-61.
4. Boumela, I., Guillemin, Y., Guérin, J.F., Aouacheria, A., The Bcl-2 family pathway in gametes and preimplantation embryos, Gynécology Obstétrique-Fertilité, 2009, 37 (9), 720-732.
5 Vaskivuo, T.E., Tapanainen, J.S., Apoptosis in the human ovary, Reprod. Biomed. Online, 2003, 6, 24-35.
6. Hussein, M.R., Haemel, A.K., Wood, G.S., p53-related pathways and the molecular pathogenesis of melanoma, Eur. J. Cancer Prev., 2003b, 12, 93-100.
7. Ferraro, E., Corvaro, M., Cecconi, F., Physiological and pathological roles of Apaf1 and the apoptosom, J. Cell Mol., 2003, 7 (1), 21-34.
8. Marchetti, P., L'apoptose: bases fondamentale set applications médicales, Gynecologie Obstetrique-Fertilité, 2005, 33 (9), 632-641.
9. Vaskivuo, T., Regulation of apoptosis in the female reproductive system, Department of Obstetrics and Gynaecology, University of Oulu, 2002
10. Hussein-Mahmoud, R., Apotosis in the ovary: molecular mechanisms, Human Reproduction Update, 2005, 11 (2), 162-178.
11. Matsuda-Minehata, F., Maeda, A., Cheng, Y., Sai, T., Gonda, H., Goto, Y., Manabe, M., Setting granulose apoptosis by death ligand-receptor signaling, J. Animal Science, 2008, 79 (1), 1-10.
12. Schmitz, S., Kirchhoff, Krammer, P.H., Regulation of death receptor-mediated apoptosis pathwys, Cell Biol., 2000, 32, 1123-1136.
13.fille://H:/New%20/Folder/Death%20Receptorshtm
14. Dharma, S.J., Kelkar, R.L., Nandedkar, T.D., Fas and Fas ligand protein and mRNA in normal and atretic mouse ovarian follicles, Reproduction, 2003, 126, 783-789.
15. Cataldo, N.A., Dumesic, D.A., Goldsmith, P.C., Jaffe, R.B., Immunolocalization of Fas and fas ligand in the ovaries of women with polycystic ovary

syndrome: relation ship to apoptosis, Human Reproduction, 2000, 15, 1889-1897.
16. Inoue, N., Maeda, A., Matsuda-Minehata, F., Fukuta, K., Manabe, N., Expression and localization of Fas ligand and Fas during atresia in porcine ovarian follicles, Journal and Reproduction and Development, 2006, 52, 723-730.
17. Porter, D.A., Vickers, S.L., Cowan, R.G., Huber, S.C., Quirk, S.M., Expression and function of Fas antigen vary in bovin granulosa and theca cell duryng ovarian follicular development and atresia, Biology and Reproduction, 2000, 62, 62-66.
18. Porter, D.A., Harman, R.M., Cowan, R.G., Quirk, S.M., Relation ship of Fas ligand expression and atresia during bovine follicle development, Reproduction, 2001, 121, 561-566.
19. Vickers, S.L., Cowan, R.G., Harman, R.M., Porter, D.A., Quirk, S.M., Expression and activity of the Fas antigen in bovine ovarian follicle cell, Biology and Reproduction, 2000, 62, 54-61.
20. Benifla, J.L., Sifer, C., Bringuier, A.F., Blanc-Layrac, G., Camus, E., Madelenat, P., Feldmann, G., Induced apoptosis and expression of related proteins in granulosa cell from womwn undergoing IVF: a preliminary study, Human Reproduction, 2002, 17, 916-920.
21. Kuranaga, E., Kanuka, H., Furuhata, Y., Yonezawa, T., Suzuki, M., Nishihara, M., Takahashi, M., Requirement of the Fas-ligand expressing luteal immune cells for regression of corpus luteum, FEBS Letters, 2000, 472, 137-142.
22. Vijay-Yadav, K., Lakshmi, K., Medhamurthy, R., Prostaglandin $F_{2\alpha}$ –mediated activation of apoptotic signaling cascades in the corpus luteum during apoptosis, J. Biol. Chem., 2005, 280 (11), 10357-10367.
23. Inoue, J., Ishida, T., Tsukamoto, N., Kobayaski, N., Naito, A., Azuma, S., Yamamoto, T., Tumor necrosis factor receptor-associater factor (TRAF) family: Adapter proteins that mediate cytokine signaling, Experimental Cell Research, 2000, 254, 14-24.
24. Wajan, H., Scheurich, P., Tumor necrosis factor receptor-associated factor (TRAF2) and its role in TNF signaling, International Journal of Biochemistry and cell Biology, 2001, 33, 19-32.
25. Prange-Kiel, J., Kreutzkamm, C., Wehrenberg, U., Rune, G.M., Role of tumor necrosis factor in preovulatory follicles of swine, Biology of Reproduction, 2001, 65, 928-935.
26. Nakayama, M., Manabe, M., Inoue, N., Matsui, T., Miyamoto, H., Changes in the exppression of the tumor necrosis factor (TNF) α, TNFα receptor (TNFR)2, TNFR-associated factor 2 in granulosa cell during atresia in pig ovaries, Biology of Reproduction, 2003, 68, 530-535.
27. Pate J. L., Landys-Keyes, P., Immune cell in the corpus luteum: friends or foes?, Reproduction, 2001, 122, 665-676.
28. Friedman, A., Weiss, S., Levy, N., Meidan, R., Role of tumor necrosis factor α and its type I receptor in luteal regression: induction of programmed cell death in bovine corpus luteum-derived endothelial cell, Biology of Reproduction, 2000, 63, 1905-1912.