Review Article

APOPTOSIS AND HUMAN REPRODUCTION

(Received 27 March, 1997)

M.A. Akman, M.D.* / E. Büyük, M.D.**

* *Specialist, Department of Obstetrics and Gynecology, Faculty of Medicine, Marmara University, Istanbul, Turkey*

** *Resident, Department of Obstetrics and Gynecology, Faculty of Medicine, Marmara University, Istanbul, Turkey.*

ABSTRACT

Apoptosis is a descriptive term to describe a type of cell death with morphological changes distinct from necrosis. It acts in a programmed manner and through this mechanism the body deletes unwanted cells without eliciting any immune response or inflammatory reaction. In human reproductive tract, recent data demonstrate that it plays an important role in some physiologic events like, the perinatal loss of germ cells, the process of follicular atresia during normal menstrual life and menopause, the regression of corpus luteum, the cyclic shedding of endometrium and some beneficial effects on the decidua during early pregnancy and later in placenta. With the further understanding of the exact mechanisms, it will guide us to solve some common clinical problems and promote the human reproductive functions.

Key Words: Apoptosis, Human reproduction

INTRODUCTION

In biological organisms, there are two fundamental types of cell death: necrosis and apoptosis. Apoptosis, meaning in ancient Greek "falling off" is a physiological process resembling mitosis that leads in a controlled fashion to cell death. It has been first described in 1972 and the body deletes unwanted cells without eliciting any immune response or inflammatory reaction through inducing this process(1).

Apoptosis is thought to be responsible for numerous physiologic and pathological events, some examples are: the programmed cell destruction during embryogenesis; hormone-dependent involution of cells in adult, as endometrial cell breakdown during the menstrual cycle, ovarian follicular atresia in the menopause, regression of the lactating breast after weaning; cell death in tumours; death of immune cells, as in developing thymus; cell deletion in proliferating cell populations, as in intestinal crypt epithelia.

Characteristics of Apoptosis

Since apoptosis is one of the basic mode of cell injury, it has to be differentiated from necrosis. Apoptosis has some unique pathologic features: dense cytoplasm and tightly packed organelles leading to cell shrinkage; chromatin condensation under the nuclear membrane, which is the most characteristic feature of apoptosis; extensive surface blebbing of the apoptotic cells leading to fragmentation into a number of membrane bound apoptotic bodies composed of cytoplasm and tightly packed organelles; phagocytosis of apoptotic cells or bodies by adjacent healthy parenchymal cells or macrophages without eliciting any inflammatory reaction (2).

The characteristic chromatin condensation yields degradation of DNA resulting DNA fragments in size multiplies of 185 to 200 base pairs that can be observed as a distinct "ladder pattern" for apoptotic cells by gel electrophoresis (3). This unique feature distinguishes cell death by apoptosis from accidental or pathologic cell death in which the DNA is randomly degraded.

Regulators Of Apoptosis

Certain genes have been shown to play a regulatory role in the induction of apoptosis, so in controlling cell fate. It is well established that members of the bcl-2 gene family function as primary determinants of the cell fate (4). This proto-oncogene inhibits apoptosis induced by hormones and cytokines and thus extends cell survival (5). Its protein, namely bcl-2 protein is known as a cell survival factor that acts by preventing cell death by apoptosis. Another oncogene, c-myc has been shown to be effective through its protein product which stimulates

apoptosis. Finally, recent data have revealed that the product of p53 tumour suppresser gene normally induces apoptosis, but when mutated or absent like in certain cancers, prolongs the cell survival (6).

Apoptosis in the Ovary

The first observations about the physiological cell death of the granulosa cells in the rabbit ovaries date back to 19th century (7). Later, in 1972 it has been shown that apoptosis is the mechanism by which granulosa cells die during follicular atresia (1). Apoptosis has been implicated in several events that occur during the ovarian life cycle: the reduction in oocyte number that occurs during fetal life, the process of follicular atresia, and the regression of corpus luteum.

Apoptosis and Perinatal Loss of Germ Cells

Primordial germ cells first appear in the wall of the yolk sac at the end of the third week of the development: they migrate to the genital ridge at the beginning of the fifth week and differentiate into oogonia, proliferate by mitosis and become invested by a layer of flat epithelial cells, forming primordial follicles and differentiating into oocytes. During the next few months, they increase rapidly in number and by the 20th week, the total number of germ cells in the ovary reaches its maximum, estimated as 7.000.000. However, a tremendous level of germ cell attrition occurs in the fetal ovary during the second and third trimesters and at the end of the fetal development, approximately 2.000.000 oocytes remain. In the mouse this attrition occurs via induction of apoptosis (8). However, it has been reported that germ cell attrition may result from inadequate levels of a somatic cell-derived growth factor (SCF), which is required for germ cell survival (9). On mice, it has been shown that loss of expression of the SCF gene results in gonadal dysgenesis and sterility and addition of SCF into primordial germ cell cultures in vitro effectively prevents apoptosis (10,11).

In a mouse study, when the bcl-2 gene expression is blocked, the number of oocytes and primordial follicles in the perinatal ovary are found to be reduced (12). The same phenotypic appearance of the ovary has been observed when the genes responsible for SCF or its receptors are not expressed (9). Thus it might be speculated that SCF may show its effect in a combined fashion with apoptosis regulatory genes, like bcl-2.

Folliculogenesis and Apoptosis

Atresia is the form of follicular degeneration in all vertebrate species. The mechanism of the follicular atresia has been reported to be through induction of apoptosis by Zeleznik et al (13). On the rat ovarian follicles and corpora lutea, they showed the presence of the Ca++/Mg++-dependent endonuclease action (which produces the oligonucleosomal DNA fragmentation in apoptosis). Later, the characteristic "ladder pattern" of DNA cleavage of the apoptosis have been used as a marker for the granulosa cell demise.

In numerous studies, many hormones and growth factors have been shown to promote or prevent apoptosis (14-16). FSH, LH/hCG, Epidermal Growth Factor (EG-F) prevent apoptosis: *in* hypophysectomized, DES-treated rats, estrogens prevented apoptosis while androgens and GnRH-agonists promoted it. In intact follicles, IGF-1, like gonadotropins, prevented apoptosis: interestingly, this action can be blocked by insulin like growth factor binding protein 3 (IGFBP-3), suggesting that IGF's mediate the effects of gonadotropins. Growth hormone and IL-1 have also been shown to prevent apoptosis.The exact mechanism by which these regulators prevent or promote apoptosis has not been clearly established but there is ample evidence that the intracellular events dictating the fate of ovarian cells would be similar to those described for the regulation of apoptosis in extragonadal cells. For instance, molecules of the bcl-2 family are expressed *in* animal and human granulosa cells and treatment of immature rat ovary with pregnant mare serum gonadotropins in vivo decreased bax mRNA expression, without affecting bcl-2 or bcl-x expression i.e.: apoptosis is prevented; whereas serum-free follicle culture increased bax but not bcl-2 expression. Furthermore, the product of the p53 tumour suppresser gene which induce the apoptosis in several cell types has also been found to play a critical role in atretic granulosa cells: p53 gene is localised to granulosa cells only in atretic follicles, its expression is decreased by serum gonadotropins (17).

Corpus Luteum Regression and Apoptosis

Regression of the corpus luteum has been suggested to be by way of apoptosis (18). Prostaglandins, especially PGF2alfa are known to increase levels of reactive 02 species in luteal cells whereas hCG, an inhibitor of apoptosis, increases activity of enzymes that detoxify free radicals to inert compounds (18,19). Oxygen-free radical accumulation, on the other hand, is known to promote apoptosis, as well as functional and structural regression of corpus luteum. In light of these data, it may be postulated that prostaglandins and hCG play key role in luteolysis, by way of apoptosis. Recent studies have provided evidence for regulated expression of bcl-2 and c-myc proteins in luteal function (20,21). Therefore, as apoptosis of

oocytes and granulosa cells, luteolysis is mediated via a pathway involving these conserved regulators of apoptosis.

Apoptosis in the Endometrium

On the endometrium, the cyclic shedding of the endometrium is suggested to be through apoptosis. The apoptosis regulating factors, namely EGF and IGF-1 were found to be parallel with estrogen levels through the menstrual cycle (22). As early as 22nd week of gastation, bcl-2 protein has been demonstrated in the endometrial epithelium and stroma (23). Additionally, bcl-2 protein shows cyclic pattern in endometrium; peaks in late follicular phase, disappears in the late secretory and menstruation period (24). This pattern suggests gonadal steroid regulation of bcl-2 in the endometrium.

Apoptosis has also been shown to play an important role in the endometrial stromal cells for the continuity of the early pregnancy. In rodent studies, it has been shown that during the trophoblast attachment, apoptosis occurs in the epithelial cells and decidua adjacent to the trophoblast, allowing direct contact between embryo and decidual cells (25). Hence, it seems to play an important role in normal trophoblastic invasion.

Apoptosis in the Placenta

As in early pregnancy, apoptotic changes have been shown to take place in syncytiotrophoblast of the term human placental villi (26). Through electron microscopy, Nelson showed the apoptotic changes in the syncytiotrophoblast layer where the fibrin type fibrinoid depositions occuring at discontinuties in the villus trophoblast. So, the apoptosis through the effects on the barrier layer, syncytiotrophoblast, seems to play another important role in placenta, probably maintaining the maternal-fetal exchange.

CONCLUSION

Apoptosis is the programmed cell death occurring throughout normal development. A better elucidation of the mechanisms of this process will enlighten our understanding of the reproductive functions, will guide us to altered treatment modalities: prevention of oocyte depletion will be favourable for premature ovarian failure patients, will prolong the reproductive years i.e. postpone the menopause age while increasing the fecundity rates and once the pregnancy is achieved, early pregnancy losses will be prevented through the effects on both endometrium and luteolysis process. Additionally, prevention of follicular atresia and death may be helpful in recruitment of larger cohort of healthy follicles during ovulation induction regimens. These and other future efforts to bridge the gap between basic science and clinical medicine are critical to ensure that the study of apoptosis in the context of ovarian function will continue to provide new and exciting information that is truly food for thought.

REFERENCES

1. *Kerr JFR, Wyllie AH, Currie AR. Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972;26:239-245.*
2. *Wyllie AH. Cell death: the significance of apoptosis. Int Rev Cytol 1980;68:251-257.*
3. *Schwartzman RA, Cidlowski JA. Apoptosis: the biochemistry and molecular biology of programmed cell death. Endocrine Rev 1993;14:133-137.*
4. *Tilly JL, Tilly KI, Kenton ML, et a. Expression of members of the bcl-2 gene family in the immature rat ovary: equine chorionic gonadotropin-mediated inhibition of granulosa cell apoptosis is associated with decreased bax and constitutive bcl-2 and bcl-xlong mRNA levels. Endocrinology 1995;136:232-241.*
5. *Korsmeyer SJ. Bcl-2: an antidote of programmed cell death. Cancer Surv 1992;15:105-108.*
6. *Wyllie AH. The genetic regulation of apoptosis. Curr Opin Genetics Dev 1995;5:97-104.*
7. *Flemming W. Uber die bildung von richtungsfiguren in saugethiereiren beim untergang Graafscher follikel. Arch Anat EntwGesch 1885;221.*
8. *Coucouvanis EC, Sherwood SW, Carswell-Crumpton C, et al. Evidence that the mechanism of prenatal germ cell death in the mouse is apoptosis. Exp Cell Res 1993;209:238-247.*
9. *Huang EJ, Manova K, Packer AI, et al. The murine steel panda mutation affects kit ligand expression and growth of early ovarian follicles. Dev Biol 1993;157:100-109.*
10. *Besmer P, Manova K, Duttlinger R, et al. The kit ligand (Steel factor) and its receptor c-kit/W: Pleiotropic roles in gametogenesis and melanogenesis. Development 1993(Suppl):125-137.*
11. *Pesce M, Farrace MG, Piacentini M, et al. Stem cell factor and leukemia inhibitory factor promote primordial germ cell survival by suppressing programmed cell death (apoptosis). Development 1993;118:1089-1094.*
12. *Ratts VS, Flaws JA, Kolp R, et al. Ablation of bcl-2 gene expression decreases the numbers of oocytes and primordial follicles established in the postnatal female mouse gonad. Endocrinology 1995;136:3665-3668.*
13. *Zeleznik AJ, Ihrig LL, Basset SG. Developmental expression of Ca++/Mg++-dependent endonuclease activity in rat granulosa and luteal*

cells. Endocrinology 1989;125:2218-2220.
14. Chun SY, Billig H, Tilly JL, et al. Gonadotropin suppression of apoptosis in cultured preovulatory follicles: mediatory role of endogenous insulin-like growth factor-I. Endocrinology 1994;135:1845-1853.
15. Tilly JL, Billig H, Kowalski KI, et al. Epidermal growth factor and basic fibroblast growth factor suppress the spontaneous on set of apoptosis in cultured rat ovarian granulosa cells and follicles by a tyrosine kinase-dependent mechanism. Mol Endocrinol 1992;6:1942-1947.
16. Tilly JL, Flaws JA, DeSanti A, et al. Role of intrafollicular growth factors in maturation and atresia of rat ovarian follicles. Biol Reprod 1995;52(Suppl):159.
17. Tilly KI, Banerjee S, Banerjee PP, Tilly JL. Expression of the p53 and Wilms' tumor suppressor genes in the rat ovary: Gonadotropin regression in vivo and immunohistochemical localisation of nuclear p53 protein to apoptotic granulosa cells of atretic follicles. Endocrinology 1995;136:1394-1402.
18. Dharmarajan AM, Goodman SB, Tilly KI, Tilly JL. Apoptosis during functional corpus luteum regression: evidence of a role for chorionic gonadotropin in promoting luteal cell survival. J Endocrinol 1994;2:295-301.
19. Carlson JC, Wu XM, Sawada M. Oxygen radicals and the control of ovarian corpus luteum function. Free Radical Biol Med 1993;14:79-84.
20. Rodgers FE, Fraser HM, Duncan WC, et al. Immunolocalisation of bcl-2 in the human corpus luteum. Human Reprod 1995;10:3242-3247.
21. Fraser HM, Lunn SF, Cowen GM, et al. Induced luteal regression in the primate: evidence for apoptosis and changes in c-myc protein. J Endocrinol 1995;147:131-137.
22. Nelson K, Takahash, T, Bossert N, et al. Epidermal growth factor replaces estrogen in the stimulation of female genital tract growth and differentiation. Proc Natl Acad Sci 1991;88:21-25.
23. Le Brun B, Warnke R, Cleary M. Expression of bcl-2 in fetal tissues suggests a role in morphogenesis. Am J Pathol 1993;142:743-753.
24. Gompel A, Sabourin J, Martin A, et al. Bcl-2 expression in normal endometrium during the menstrual cycle. Am J Pathol 1994;144:1195-1202.
25. Gu Y, Jow G, Moulton B, et al. Apoptosis in decidual tissue regression and reorganisation. Endocrinology 1994;135:1272-1279.
26. Nelson DM. Apoptotic changes occur in syncytiotrophoblast of human placental villi where fibrin type fibrinoid is deposited at discontinuities in the villus trophoblast. Placenta 1996;17:387-391.