Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 66 (2003) 1393–1401
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Pleiotropic anti-apoptotic activity of glucocorticoids in ovarian follicular cells

R. Sasson, A. Amsterdam*

Department of Molecular Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel

Received 6 April 2003; accepted 7 May 2003

Abstract

Glucocorticoids (GC) such as hydrocortisone and dexamethasone (DEX) protect steroidogenic granulosa cells against apoptosis induced by serum deprivation, cAMP, tumor necrosis factor α stimulation or p53 activation. The protective effects were evident both in primary rat and human granulosa cells, which comprise the main population of the ovarian follicular cells, as well as in steroidogenic granulosa cell lines established in our laboratory. A correlation between the expression of Bcl-2 protein and protection against apoptosis induced by DEX was found in granulosa cell lines expressing various levels of Bcl-2. Incubation with DEX leads to development of a rigid network of actin cytoskeleton and increased incidence of adherence and gap junctions. Higher content of connexin 43 and total cadherins were found in GC stimulated cells compared to non-stimulated, suggesting that cell contact and intracellular communication contribute to the DEX induced resistance to apoptotic signals. Activation by DEX of MAPK and Akt/PKB but not p38 supported the view of a pleiotropic action of GC against apoptotic signals. Granzyme B, a protease characteristic for induction of apoptosis by T-cytotoxic lymphocytes and natural killer cells, was expressed and augmented during stimulation of apoptosis in the granulosa cells, and its synthesis and activation was blocked by DEX. It is concluded that GC exerted their anti-apoptotic effects in granulosa cells by multiple characteristic pathways. Moreover, the presence of endogenous granzyme B in granulosa cells suggest a novel intrinsic alternative apoptotic pathway that was earlier reported to be mediated uniquely by T-cytotoxic lymphocytes and natural killer cells. The anti-apoptotic effect of GC may play an important role in the healing process of the ovulatory follicle subsequent to follicular rupture and its rapid conversion to an active corpus luteum.
© 2003 Elsevier Inc. All rights reserved.

Keywords: Steroidogenic granulosa cells; Bcl-2; Dexamethasone; Granzyme B; p53; TNFα

1. Introduction

Ovarian cell death is an essential process for the homeostasis of ovarian function in human and other mammalian species. It ensures the selection of the dominant follicle and the demise of excess follicles. In turn, this process minimizes the possibility of multiple embryo development and assures the development of few but healthy embryos (reviewed in [1–3]). Although several hundred thousands of primordial and primary follicles are present in the mammalian ovary before puberty, only very few will fully mature and ovulate. The others will be eliminated by atresia, a process that exhibits both the biochemical and morphological features of programmed cell death, which includes blebbing of the cell membrane and rearrangement of the actin cytoskeleton, followed by DNA degradation [3–6]. Moreover, the demise of the old corpus luteum after ovulation and formation of a new corpus luteum, assures the prompt rise and fall of progesterone secretion during the estrous/menstrual cycle and was also characterized as programmed cell death [7–11].

GC are widely used as immunosuppressive and anti-inflammatory agents. They have been shown to inhibit the expression of cytokines, adhesion molecules and enzymes involved in the inflammatory process [12]. The effect of GC on ovarian function *in vivo* is not completely understood. However, in cultured granulosa cells obtained from *in vitro* fertilization (IVF) patients and from rat preovulatory follicles, GC were found to enhance gonadotropin/cAMP induced steroidogenesis [13–15] and protected efficiently against ovarian apoptotic signals [16], although

* Corresponding author. Tel.: +972-8-9343713; fax: +972-8-9344125.
E-mail address: abraham.amsterdam@weizmann.ac.il (A. Amsterdam).
Abbreviations: CX-43, connexin-43; DEX, dexamethasone; GC, glucocorticoids; TNFα, tumor necrosis factor α.

0006-2952/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S0006-2952(03)00489-1

in many cell systems GC are potent inducers of apoptosis (reviewed in [17,18]).

Regulation of apoptotic signaling is achieved in general by expression of distinct protein families, such as the Bcl-2 family. The Bcl-2 family consists of two subfamilies; pro-apoptotic members such as Bax, Bad, Bik, Bim, or Bcl-x_S which initiate or promote apoptotic signals, and anti-apoptotic members such as Bcl-2, Bcl-x_L, Mcl-1, or A1 which block the activation of effector caspases, such as caspase-3 and caspase-2, which transduce the apoptotic signals (reviewed in [19]). Bcl-2, present in the outer mitochondria membrane, has been suggested to block apoptosis by inhibiting the release from mitochondria of apoptosis-inducing factors, such as cytochrome *c* [20,21]. Cytochrome *c* serves as an important cofactor for the activation of Apaf-1, which is located in the cytoplasm and is able to activate caspase-9 [22]. Phosphorylation of Bcl-2 is required for its proteasome-dependent degradation [23]. Although the role of Bcl-2 in ovarian function is not fully elucidated, a growing number of reports have implicated it and related members of this gene family in ovarian cell death. For example, the *bcl-2* gene has been detected in the ovary of many species [24–26] and ablation of functional Bcl-2 through targeted disruption of the gene in mice (''knock out'') leads to significantly fewer oocytes and primordial follicles in the postnatal ovary [27]. Bcl-2 was recently found also to be involved in leptin protection against follicular cell death [28].

Distinct pharmacological modulation of apoptosis is achieved by GC, such as DEX and hydrocortisone. Depending on cell type, DEX either elicits apoptosis, in thymocytes and lymphocytes, or exerts potent anti-apoptotic effects in

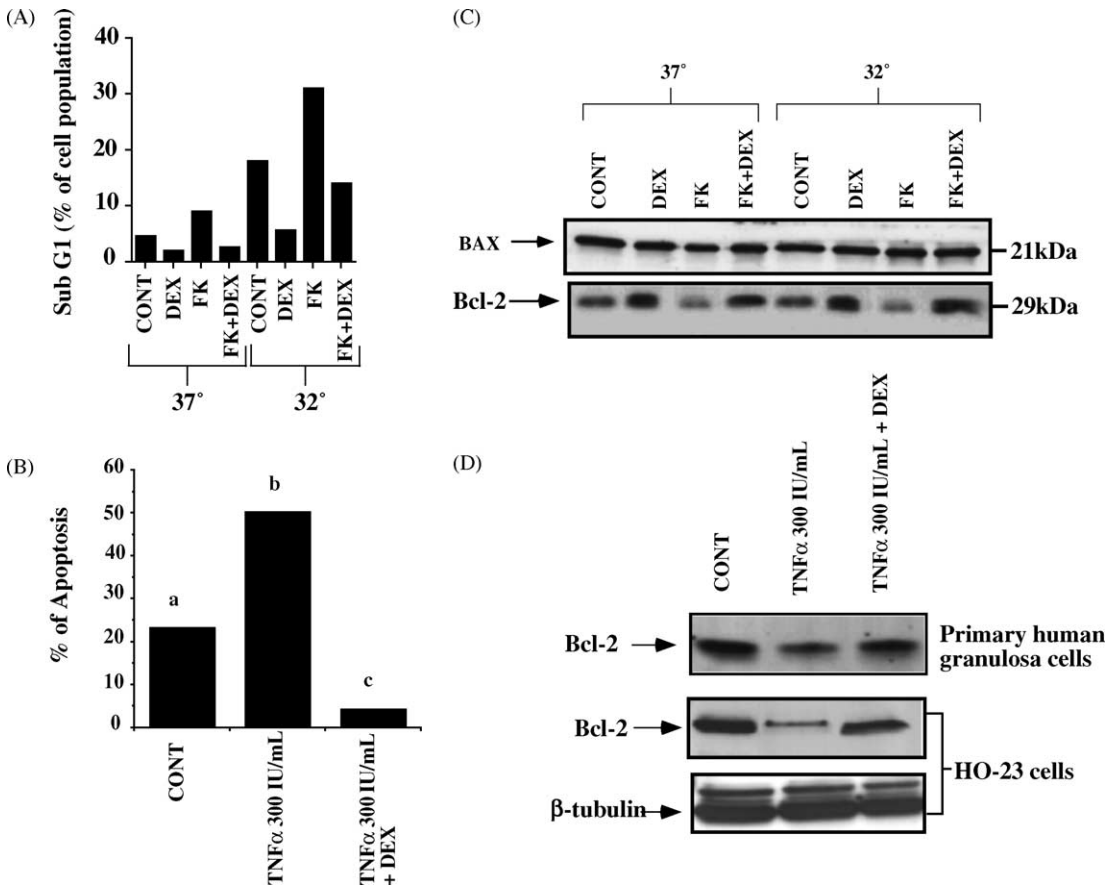

Fig. 1. Modulation of Bcl-2 and BAX levels in granulosa cells. (A) Rat granulosa cell line RGSP53 was established by triple transfection of primary rat granulosa cells, with SV40 DNA, Ha-ras and a temperature-sensitive (Ts) mutant of p53 (p53val135) [60]. Shifting the temperature of growth from 37 to 32° leads to manifestation of wild-type p53 activity [45,61]. RGSP53-10 cells were cultured for 24 hr in medium containing 5% serum at 37° for 24 hr. The medium was replaced with serum-free medium alone (CONT), or containing 20 μM forskolin (FK), 100 nM DEX or both (FK + DEX) at 37°. At the end of 24 hr incubation, an additional 5 hr incubation was carried out in parallel at 32 or 37°. Cells were fixed with methanol, stained with propidium iodide, and incidence of apoptosis (sub G1 fraction) in the different treatments was examined by FACS. (A′) Western blot analysis was performed on cell lysates obtained after identical treatments as in 'A' using specific antibodies to BAX and Bcl-2. (B) Primary cultures of human granulosa cells were incubated with DMEM/F12 medium containing 5% fetal calf serum for 7 days to release them from desensitization [14] and subsequently in serum-free medium with the indicated stimulants for 24 hr at 37°. The culture medium was replaced with serum-free medium in the absence of stimulants (CONT), or in the presence of 300 IU/mL TNFα alone or with 100 nM of DEX for 24 hr. Percentage of apoptosis was determined as % of positively 'stained nuclei by TUNEL per total nuclei stained by DAPI. Data are mean of triplicate plates ± SEM; 'a' is different from 'b' and 'c' $P < 0.01$. (B′) Cell lysates were prepared from identical treatments, as in 'B', and Western blot analysis was performed using specific antibodies to Bcl-2 and β-tubulin. Similar treatments of immortalized granulosa cells showed essentially similar results. HO-23 is a steroidogenic human granulosa cell line established by triple transfection with SV40DNA, Ha-Ras and Val 135 p53 [60] (modified by permission from [15,16]).

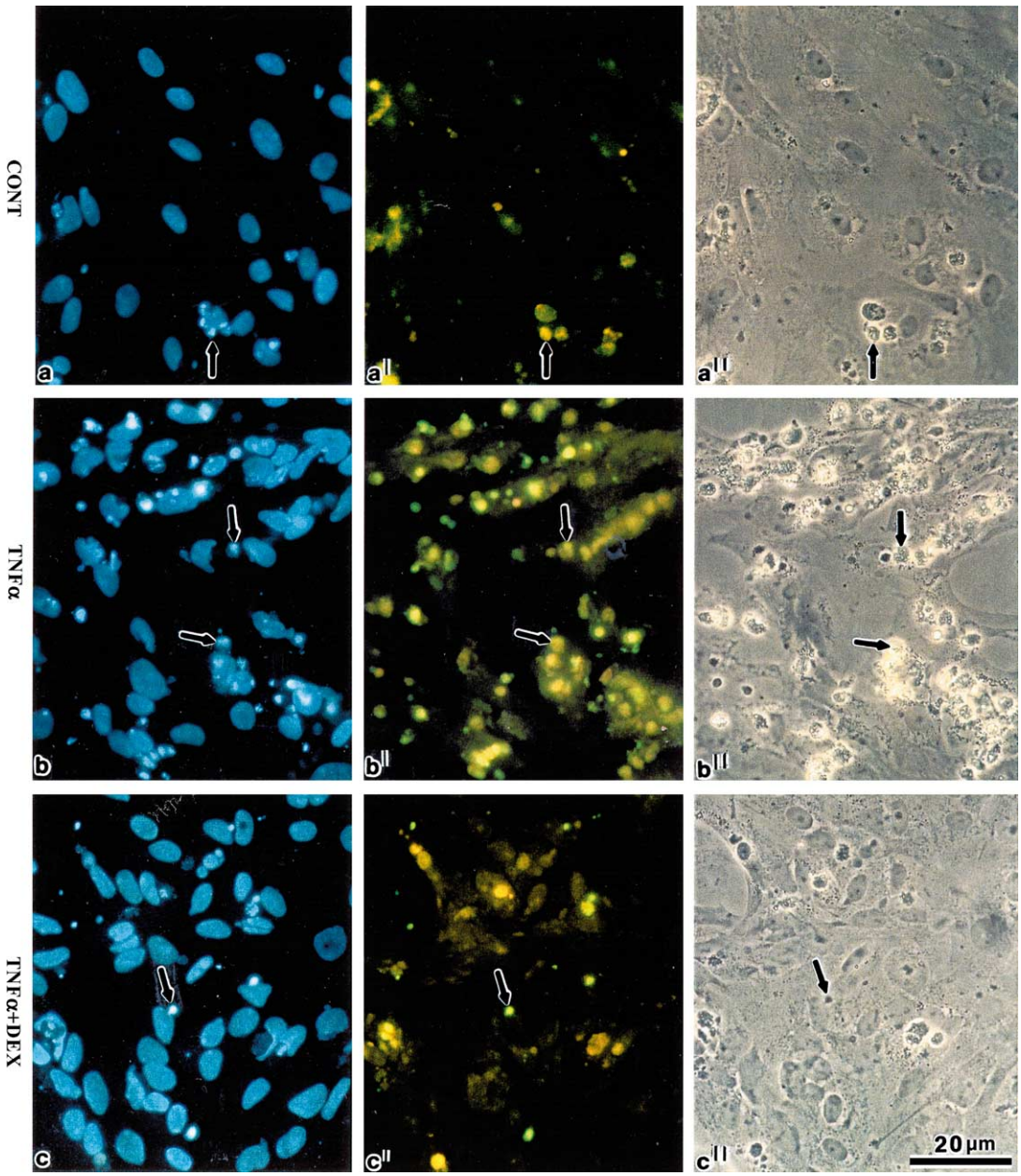

Fig. 2. Attenuation of TNFα-induced apoptosis by dexamethasone in rat preovulatory granulosa cells. Primary cultures were obtained from PMSG-stimulation immature female rats [15]. Cells were incubated with no stimulants in DMEM/F12 serum free medium (CONT; a, a′, a″), TNFα (300 IU/mL) (b, b′, b″), or with TNFα + 100 nM of DEX (c, c′, c″) for 24 hr at 37°. At the end of the incubation period cells were fixed with 4% *para*-formaldehyde and stained with DAPI (a, b, c) and TUNEL (a′, b′, c′). The same fields were also visualized by phase contrast microscopy (a″, b″, c″). Arrows are indicating apoptotic nuclei (by permission from [15]).

various cell types such as, lung epithelial cells [29,30], human neutrophils [31], rat hepatoma cells [32] and glomerular endothelial cells [33,34]. Although DEX has been shown to increase Bcl-2 expression [16] and to suppress up-regulation of pro-apoptotic members of the Bcl-2 family such as Bcl-x_S and Bak [34,35], as well as to prevent the down-regulation of Bcl-x_L expression, and the activation of caspase-3 in response to TNFα [34,36], the anti-apoptotic mechanism of DEX still remains to be fully elucidated.

In our research we present evidence that GC serve as very potent inhibitors of apoptosis of granulosa cells which comprise the main bulk of the ovarian somatic cells when in most cases apoptosis of the ovarian follicle initiates in the inner layers of granulosa cells facing the follicular antrum [14–16]. We have discovered that GC prevent apoptosis induced by cAMP stimulation, p53 activation (Fig. 1) and TNFα stimulation (Fig. 2). These effectors may play also an important role in the regulation of ovarian cell death *in vivo*.

1.1. Role of Bcl-2

We demonstrated that DEX suppressed apoptosis in primary human granulosa cells and in immortalized human granulosa cell lines via modulation of Bcl-2 expression

[14,16]. This phenomenon was consistent for p53, cAMP and TNFα stimulation of apoptosis both in primary and immortalized granulosa cells. The anti-apoptotic effect of DEX was evident at low concentrations of the hormone (ED_{50}: 7 nM) and was blocked by the specific inhibitor RU486, suggesting that the effect of GC is mainly via a cytosolic receptor [16]. Interestingly, we have recently discovered that GC enhance the intracellular level of Bcl-2 protein without affecting the level of its mRNA. Since DEX affects moderately the turnover of Bcl-2, we suggest that DEX increases the translation rate of Bcl-2 [37,38]. Higher concentration of polyribosomes in DEX treated cells compared to non-treated cells may facilitate higher rate of mRNA translation in Dex-treated cells compared to non-stimulated cells [39] (and our unpublished observations). Experiments using cell lines expressing different amounts of Bcl-2 show that the protective effect of DEX against apoptosis, when extremely low levels of the hormone is used, is evident only in cells that express high levels of Bcl-2, suggesting intrinsic connection between Bcl-2 intracellular levels and survival activity of DEX (Fig. 3), although other mechanisms such as increasing of cell–cell contact and intercellular communication may also contribute significantly to DEX protection against apoptosis in granulosa cells. We found that DEX did not change BAX levels (Fig. 1). We therefore suggest that Bcl-2 plays a dominant role among this gene family in protecting granulosa cells against apoptosis. DEX and hydrocortisone were found to protect against TNFα induction of apoptosis both in primary and immortalized human and rat granulosa cells [15,40]. TNFα dramatically reduced intracellular levels of Bcl-2 while DEX abrogates this reduction (Fig. 1). It is therefore suggested that GC prevent apoptosis via blocking the degradation of this survival factor.

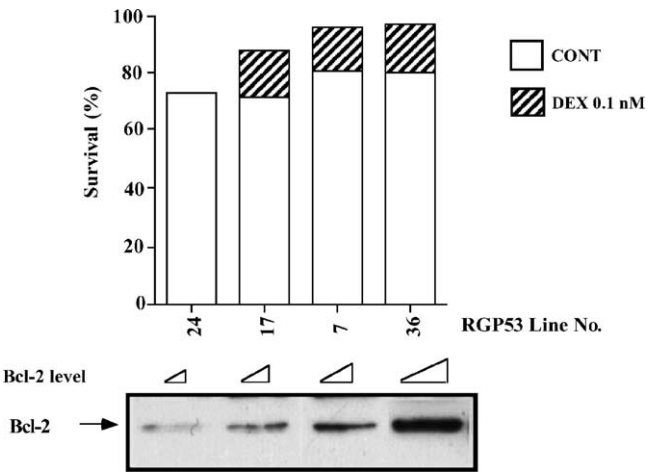

Fig. 3. Inhibition of apoptosis by DEX in different RGP53 lines. Cells were cultured for 24 hr in medium containing 5% serum at 37°. The culture medium were replaced with serum-free medium alone or with 0.1 nM of DEX. Cultures were stained with propidium iodide and processed for FACS analysis. Data of survival, 100% − sub-G1 fraction, are means from duplicate plates that deviated by <10% of their mean. Cell lysates were prepared and Western blot analysis was performed using specific antibodies to Bcl-2. Triangles refer to the basal Bcl-2 levels of the different RGP53 lines.

1.2. Role of granzyme B

We have recently discovered that granulosa cells express granzyme like proteins [41,42]. Using antibodies to granzyme B and the immunoflourescent technique reveal accumulation of granzyme B in small granules. Western blot analysis demonstrate that the 31 kDa protein of granzyme B is processed to yield a 27–28 kDa active protease when cells are stimulated to undergo apoptosis by high levels of cAMP [41]. It seems that this pathway can bypass mitochondria destruction during initial steps of apoptosis and allows steroidogenesis to go on until total collapse of the cell is occurred. Interestingly, GC almost completely block granzyme B production, and these observations suggest that GC can block multiple pathways that are involved in apoptosis in granulosa cells (Fig. 4).

1.3. Protease involvement in DEX inhibition of apoptosis

Activation of caspase-3 is known to participate in granulosa cell apoptosis induced by gonadotropin starvation [43,44]. Its activation is evident by cleavage of intact 37 kDa protein to form a 17 kDa fragment. Induction of apoptosis by forskolin, which elevates intracellular cAMP, did not show activation of caspase-3 (Fig. 5). In contrast, induction of apoptosis by activation of p53 (shifting the growth temperature from 37 to 32°) [16,45] clearly activated caspase-3, while forskolin at 32° did not further stimulate caspase-3 activation. Notably, DEX abolished the cleavage of 32 kDa caspase-3 (Fig. 5). A similar behavior was found for the cleavage of poly-ADP ribose polymerase (PARP), a 113 kDa nuclear protein, that binds specifically at DNA strand breaks and is a substrate for specific caspases (such as 3 and 7) which cleave PARP to an 89 kDa protein thus serving as a marker for inducible apoptosis [46].

These data suggest different pathways for cAMP- compared to p53-induced apoptosis, which could explain the additive effect of p53- and cAMP-induced apoptosis. Also the data suggest that the anti-apoptotic effect of DEX could be exerted upstream of the activation of caspase-3, as well as down stream since DEX protects against p53 induced activation of caspase-3 and PARP. Alternative pathways regulating apoptosis in granulosa cells were recently discovered [41,42]. The contribution of caspase-3 to this process is probably depend on the nature of the pro-apoptotic signal.

1.4. Role of cell contacts and intercellular communication

We demonstrated that GC elevated expression of cadherins and CX-43 which stabilized cell contact and

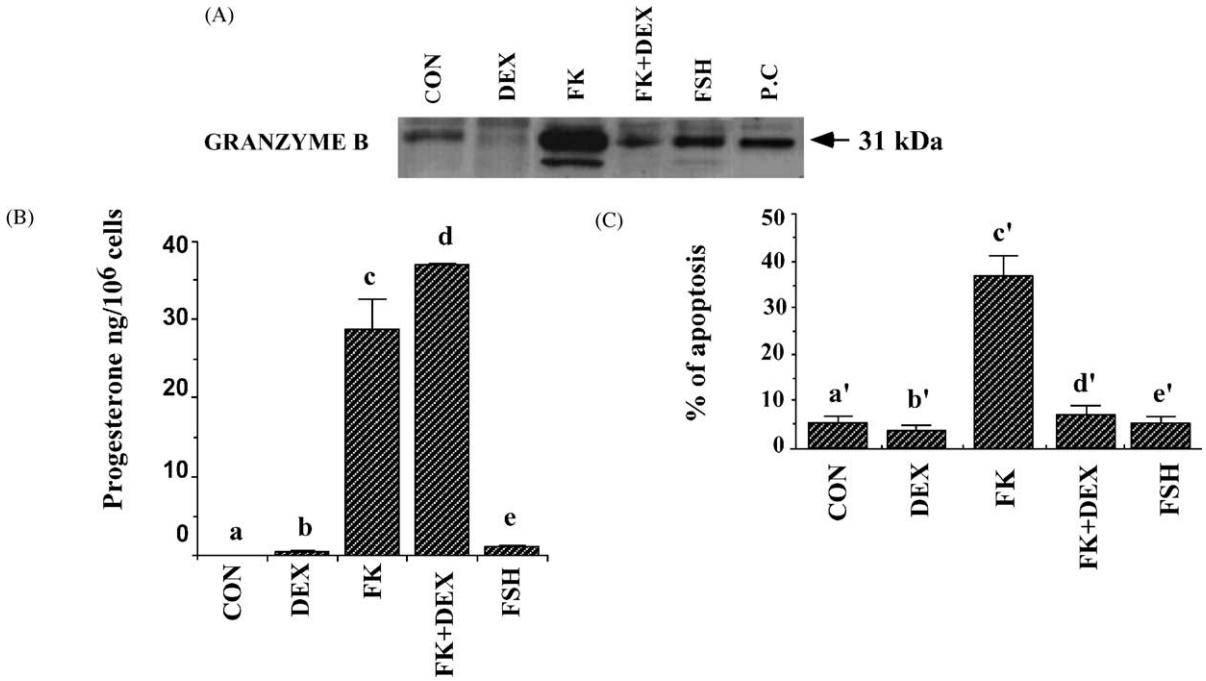

Fig. 4. Down-regulation by DEX of granzyme B-like protein induced by cAMP in rFSH-17 cells. (A) Western blot analysis of granzyme B without stimulation (CONT), following 24 hr of stimulation by hFSH and subsequently with 100 nM of DEX or 50 μM of forskolin (FK) or FK + DEX for an additional 24 hr. P.C: positive control of B3Z T cells [62]. (B) Progesterone release to the medium (measured by RIA) following the same stimulation, showing in 'A'. (C) Percentage of apoptosis was measured by FACS analysis following the same stimulation as in 'A' and subsequent methanol fixation. Data in 'B' are means ± SEM of triplicate plates: a, b < c, d; a < e, $P < 0.05$; c′ > a′, b′, d′, e′, $P < 0.01$.

intercellular communication, respectively [14,47]. Cadherins stabilize adherence type junctions. It was recently suggested that down-regulation of N-cadherin is associated with granulosa cell apoptosis, since N-cadherin mediated granulosa cell signaling may play a central role in follicular and luteal cell survival [9]. Indeed, aggregated granulosa cells were more resistant to apoptotic signals than single cells [48]. We showed that expression of cadherin is elevated following DEX stimulation concomitantly with enlargement and increased incidence of adherence junctions [47]. Therefore, we conclude that the anti-apoptotic effect of GC involves enhancement of cell contact. We have previously demonstrated that primary granulosa cells grown on extracellular matrix or in the presence of basic fibroblast growth factor (bFGF) enhance cell contact and change cell shape and organization of the actin cytoskeleton correlated with inhibition of apoptosis [49]. Therefore, paracrine and endocrine factors, which can independently elevate cell contact and organization of the actin cytoskeleton may stabilize the cells against apoptotic signals [45].

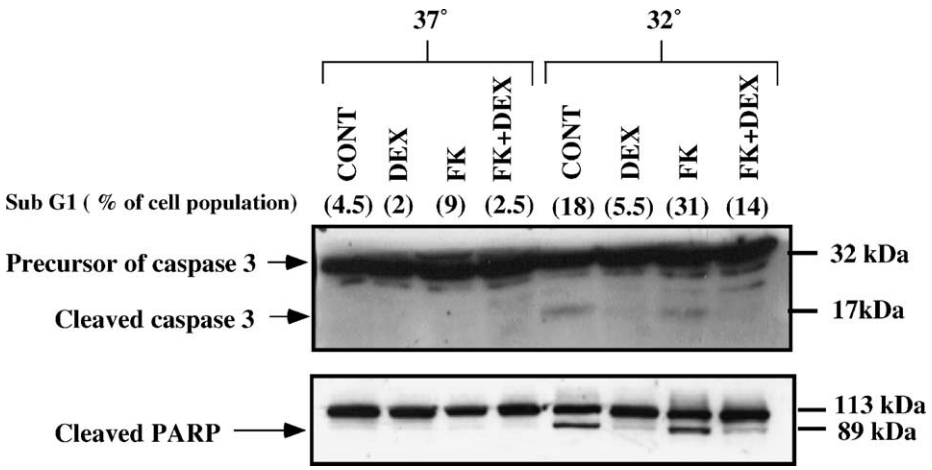

Fig. 5. DEX inhibition of caspase-3 and PARP cleavage induced by p53 in RGSP53-10 granulosa cells. Cells were stimulated for 24 hr in medium containing 5% serum at 37°. The medium was then replaced with serum free medium in the absence of stimulants (CONT) or in the presence of 20 μM forskolin (FK), 100 nM DEX or both (FK + DEX) for 24 hr at 37°. At the end of the second 24 hr incubation an additional incubation for 5 hr was carried out in parallel at 32 or 37°. Western blot analysis was performed on cell lysates using specific antibodies to caspase-3 and PARP. The incidence of apoptosis (sub G1 fraction) were measured by FACS analysis.

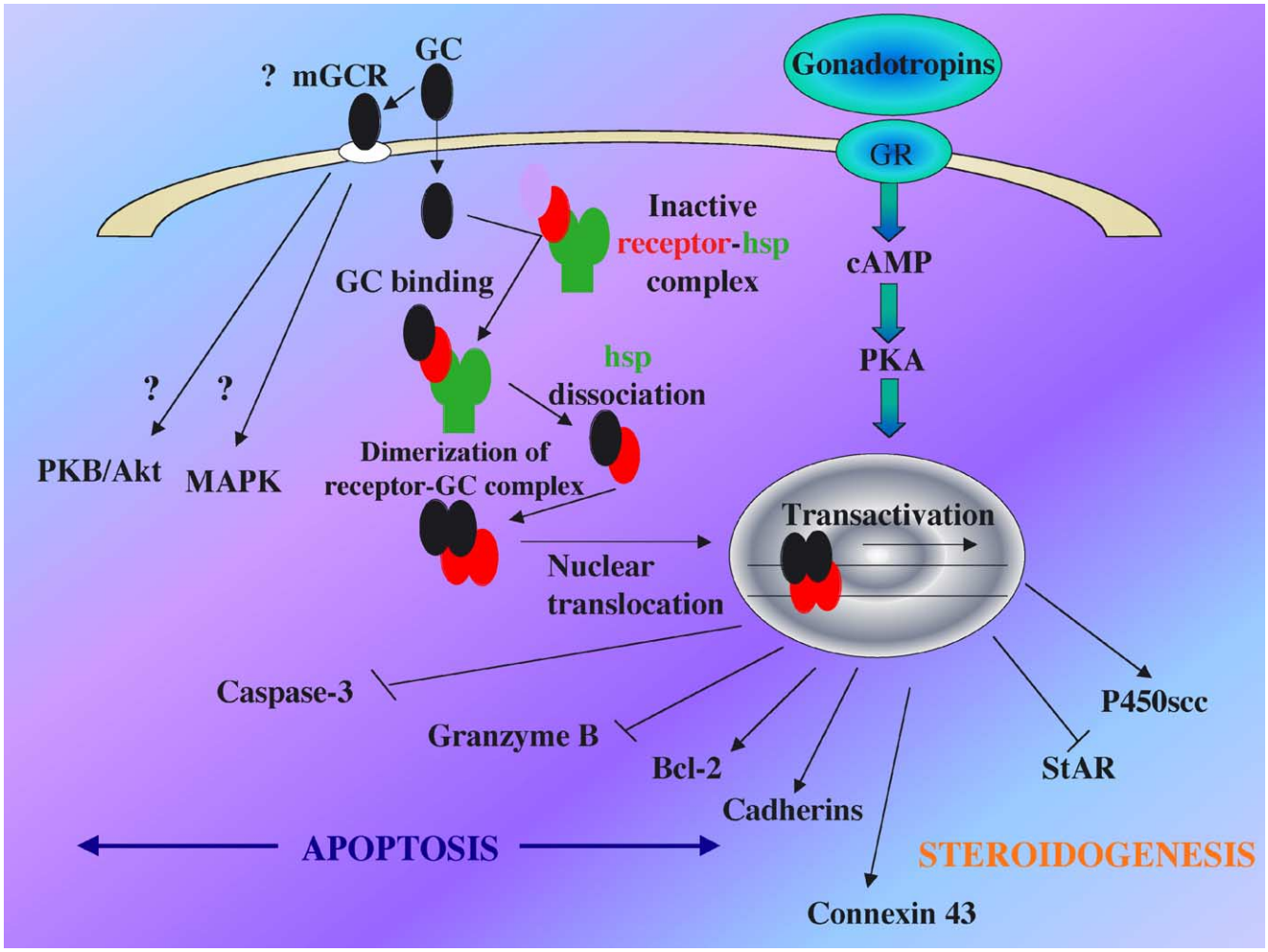

Fig. 6. Multiple pathways regulating steroidogenesis and apoptosis by GC in granulosa cells. Abbreviations: GC, glucocorticoids; mGCR, a putative membrane glucocorticoid receptor; MAPK, mitogen activated protein kinase; PKB, protein kinase B; StAR, steroidogenic acute regulatory protein; GR, gonadotropic hormone receptor; P450scc, cytochrome p450 side cleavage chain; hsp, heat shock protein 90.

Interestingly, DEX was found to up regulate cadherin expression in human fibrosarcoma cells [50].

We found that DEX enhanced CX-43 expression and appearance of intact gap junctions while cAMP also enhanced expression of CX-43, but a high proportion of the gap junctions seemed to be internalized [14]. Therefore, integrity of the junction itself rather than the concentration of its building blocks seems as important determinant in preventing apoptosis. In hepatocytes DEX enhanced formation of gap junction and expression of connexin-32 with no change in the expression of CX-43 [51]. Thus, modulation of CX-43 expression by GC is cell specific.

1.5. Role of protein phosphorylation

In our study we demonstrated a rapid activation (phosphorylation) of MAPK and PKB/Akt by DEX.[1] Activation of ERK 1/2 has been proposed to mediate an anti-apoptotic effect in ovarian granulosa cells [52,53] and activation of the Akt/protein kinase B signaling pathway has been associated with granulosa cell survival [54,55]. By contrast, in multiple myeloma cells, DEX-induced apoptosis was associated with a significant decrease in the activities of MAPK [56]. Thus, the mechanism by which GC inhibit apoptosis probably depends on activation of MAPK while induction of apoptosis in other systems involves MAPK inhibition [56]. We found that DEX can induce Akt phosphorylation; this can explain at least in part the mechanism of DEX in inhibition of apoptosis. In contrast, in myoblasts, where DEX induces apoptosis, Singleton *et al.* [57] found that DEX inhibited the up-regulation of phospho-Akt induced by IGF-I. This effect correlated with an increased expression of the p85α subunit of PI3K, which is a negative regulator of PI3K activity, and therefore reduces Akt activity [57]. It was also found recently that E-cadherin-mediated cell contact promotes Akt kinase activity, which in turn, inhibits caspase-3 activation and thereby maintains immortalized granulosa cells viability [58]. These results are in line with our observations that DEX causes an elevation of cadherins and phospho-Akt levels.

[1] Sasson and Amsterdam, unpublished observations.

1.6. Possible physiological role of the anti-apoptotic effect of glucocorticoids in ovarian homeostasis

It was recently hypothesized that GC serve an anti-inflammatory role during ovulation thereby, promoting rapid healing of the wound left by follicular rupture in anticipation of the next ovulatory cycle [59]. Our investigation may deepen our understanding of the mechanism by which GC exert their anti-inflammatory action in the ovary. Our recent finding that GC synergize with gonadotropin/cAMP stimulation of progesterone production [15,16,60] suggest that the anti-inflammatory action of GC is exerted by two complementary mechanisms: on one hand, they induce death of cells that provoke the inflammation, and one the other hand, they protect the resident cells of the inflamed tissue by arresting apoptotic signals and by stimulating post-ovulatory steroidogenesis [17]. Deepening the knowledge on the cellular and molecular mechanisms of the diverse effects of GC in controlling cell death and differentiation of ovarian follicular cells may facilitate a more comprehensive use of these steroid hormones in controlling inflammation and possibly preventing apoptosis in some degenerative diseases.

2. Conclusions

GC receptors are expressed in granulosa cells, which implies the potential for direct action in the ovary. Our research demonstrates a direct effect of GC on steroidogenesis and protection against apoptosis induced by serum deprivation, cAMP, p53 and TNFα in granulosa cells, the main population of somatic cells in the ovarian follicle. Enhancement of steroidogenesis by GC is due to up-regulation of the cytochrome P450scc [13], while protection against apoptosis by GC was due to a concerted stimulatory effect on Bcl-2 expression, as well as, on expression of cadherins and connexin-43, the main building blocks of adherence and gap junctions (Fig. 6). Thus, the integrity of cell contact and intracellular communication plays an important role in the protective effect of GC against apoptosis. Stimulation of apoptosis in granulosa cells involved activation of caspase-3, PARP cleavage and activation of a novel intrinsic apoptotic pathway modulated by enhancement of synthesis and cleavage of granzyme B (Fig. 6). All of these pathways were dramatically attenuated by GC. The stimulatory effect of GC on steroidogenesis and the inhibitory effect of GC on apoptosis in immortalized cells were similar to primary cells, suggesting that the immortalized cells can serve as a useful system to analyze the effects of GC in cells that are closely related to normal granulosa cells. It is concluded that GC exerted their anti-apoptotic effects in granulosa cells by multiple characteristic pathways. Moreover, the involvement of endogenous granzyme B in granulosa cell apoptosis could explain an apoptotic pathway that does not lead, at the initial stages, to the loss of mitochondrial structure and steroidogenic function. Thus, the inhibitory effect of GC on granulosa cell apoptosis may serve as an important regulator of ovarian steroidogenesis and ovarian cell death.

Acknowledgments

We thank Dr. Fortuna Kohen for helpful discussion. This work was supported by grants from the Yad Avraham Center for Cancer Research and the Center for Scientific Excellence Research supported by a grant from La Fondation Raphael et Regina Levy at the Weizmann Institute of Science, Rehovot, Israel. A.A. is the incumbent of the Joyce and Ben B. Eisenberg professorial Chair of Molecular Endocrinology and Cancer Research at the Weizmann Institute of Science.

References

[1] Amsterdam A, Selvaraj N. Control of differentiation, transformation, and apoptosis in granulosa cells by oncogenes, oncoviruses, and tumor suppressor genes. Endocr Rev 1997;18(4):435–61.
[2] Amsterdam A, Dantes A, Hosokawa K, Schere-Levy CP, Kotsuji F, Aharoni D. Steroid regulation during apoptosis of ovarian follicular cells. Steroids 1998;63(5/6):314–8.
[3] Amsterdam A, Gold RS, Hosokawa K, Yoshida Y, Sasson R, Jung Y, Kotsuji F. Crosstalk among multiple signaling pathways controlling ovarian cell death. Trends Endocrinol Metab 1999;10(7):255–62.
[4] Hurwitz A, Adashi EY. Ovarian follicular atresia as an apoptotic process: a paradigm for programmed cell death in endocrine tissues. Mol Cell Endocrinol 1992;84(1/2):C19–23.
[5] Tilly JL, Kowalski KI, Johnson AL, Hsueh AJ. Involvement of apoptosis in ovarian follicular atresia and postovulatory regression. Endocrinology 1991;129(5):2799–801.
[6] Tilly JL, Kowalski KI, Schomberg DW, Hsueh AJ. Apoptosis in atretic ovarian follicles is associated with selective decreases in messenger ribonucleic acid transcripts for gonadotropin receptors and cytochrome P450 aromatase. Endocrinology 1992;131(4):1670–6.
[7] Amsterdam A, Keren-Tal I, Aharoni D. Cross-talk between cAMP and p53-generated signals in induction of differentiation and apoptosis in steroidogenic granulosa cells. Steroids 1996;61(4):252–6.
[8] Keren-Tal I, Suh BS, Dantes A, Lindner S, Oren M, Amsterdam A. Involvement of p53 expression in cAMP-mediated apoptosis in immortalized granulosa cells. Exp Cell Res 1995;218(1):283–95.
[9] Makrigiannakis A, Coukos G, Christofidou-Solomidou M, Gour BJ, Radice GL, Blaschuk O, Coutifaris C. N-cadherin-mediated human granulosa cell adhesion prevents apoptosis: a role in follicular atresia and luteolysis. Am J Pathol 1999;154(5):1391–406.
[10] Tilly JL. The molecular basis of ovarian cell death during germ cell attrition, follicular atresia. Front Biosci 1996;1:d1–d11.
[11] Aharoni D, Dantes A, Oren M, Amsterdam A. cAMP-mediated signals as determinants for apoptosis in primary granulosa cells. Exp Cell Res 1995;218(1):271–82.
[12] Barnes PJ, Adcock I. Anti-inflammatory actions of steroids: molecular mechanisms. Trends Pharmacol Sci 1993;14(12):436–41.
[13] Barkan D, Jia H, Dantes A, Vardimon L, Amsterdam A, Rubinstein M. Leptin modulates the glucocorticoid-induced ovarian steroidogenesis. Endocrinology 1999;140(4):1731–8.
[14] Sasson R, Amsterdam A. Stimulation of apoptosis in human granulosa cells from *in vitro* fertilization patients and its prevention by

dexamethasone: involvement of cell contact and bcl-2 expression. J Clin Endocrinol Metab 2002;87(7):3441–51.
[15] Sasson R, Winder N, Kees S, Amsterdam A. Induction of apoptosis in granulosa cells by TNF alpha and its attenuation by glucocorticoids involve modulation of Bcl-2. Biochem Biophys Res Commun 2002; 294(1):51–9.
[16] Sasson R, Tajima K, Amsterdam A. Glucocorticoids protect against apoptosis induced by serum deprivation, cyclic adenosine 3′,5′-monophosphate and p53 activation in immortalized human granulosa cells: involvement of Bcl-2. Endocrinology 2001;142(2):802–11.
[17] Amsterdam A, Sasson R. The anti-inflammatory action of glucocorticoids is mediated by cell type specific regulation of apoptosis. Mol Cell Endocrinol 2002;189(1/2):1–9.
[18] Evans-Storms RB, Cidlowski JA. Regulation of apoptosis by steroid hormones. J Steroid Biochem Mol Biol 1995;53(1–6):1–8.
[19] Zamzami N, Brenner C, Marzo I, Susin SA, Kroemer G. Subcellular and submitochondrial mode of action of Bcl-2-like oncoproteins. Oncogene 1998;16(17):2265–82.
[20] Chao DT, Korsmeyer SJ. BCL-2 family: regulators of cell death. Annu Rev Immunol 1998;16:395–419.
[21] Waterhouse NJ, Ricci JE, Green DR. And all of a sudden it's over: mitochondrial outer-membrane permeabilization in apoptosis. Biochimie 2002;84(2/3):113–21.
[22] Wang X. The expanding role of mitochondria in apoptosis. Genes Dev 2001;15(22):2922–33.
[23] Chadebech P, Brichese L, Baldin V, Vidal S, Valette A. Phosphorylation and proteasome-dependent degradation of Bcl-2 in mitotic-arrested cells after microtubule damage. Biochem Biophys Res Commun 1999;262(3):823–7.
[24] Johnson AL, Bridgham JT, Witty JP, Tilly JL. Expression of bcl-2 and nr-13 in hen ovarian follicles during development. Biol Reprod 1997;57(5):1096–103.
[25] Rodger FE, Fraser HM, Duncan WC, Illingworth PJ. Immunolocalization of bcl-2 in the human corpus luteum. Hum Reprod 1995; 10(6):1566–70.
[26] Tilly JL, Tilly KI, Kenton ML, Johnson AL. 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-x long messenger ribonucleic acid levels. Endocrinology 1995;136(1):232–41.
[27] Ratts VS, Flaws JA, Kolp R, Sorenson CM, Tilly JL. Ablation of *bcl-2* gene expression decreases the numbers of oocytes and primordial follicles established in the post-natal female mouse gonad. Endocrinology 1995;136(8):3665–8.
[28] Almog B, Gold R, Tajima K, Dantes A, Salim K, Rubinstein M, Barkan D, Homburg R, Lessing JB, Nevo N, Gertler A, Amsterdam A. Leptin attenuates follicular apoptosis and accelerates the onset of puberty in immature rats. Mol Cell Endocrinol 2001;183(1/2):179–91.
[29] Kull Jr FC. Reduction in tumor necrosis factor receptor affinity and cytotoxicity by glucocorticoids. Biochem Biophys Res Commun 1988;153(1):402–9.
[30] Wen LP, Madani K, Fahrni JA, Duncan SR, Rosen GD. Dexamethasone inhibits lung epithelial cell apoptosis induced by IFN-gamma and Fas. Am J Physiol 1997;273(5 Pt 1):L921–9.
[31] Kato T, Takeda Y, Nakada T, Sendo F. Inhibition by dexamethasone of human neutrophil apoptosis *in vitro*. Nat Immun 1995;14(4): 198–208.
[32] Yamamoto M, Fukuda K, Miura N, Suzuki R, Kido T, Komatsu Y. Inhibition by dexamethasone of transforming growth factor beta1-induced apoptosis in rat hepatoma cells: a possible association with Bcl-xL induction. Hepatology 1998;27(4):959–66.
[33] Messmer UK, Winkel G, Briner VA, Pfeilschifter J. Glucocorticoids potently block tumour necrosis factor-alpha- and lipopolysaccharide-induced apoptotic cell death in bovine glomerular endothelial cells upstream of caspase 3 activation. Br J Pharmacol 1999;127(7): 1633–40.
[34] Messmer UK, Winkel G, Briner VA, Pfeilschifter J. Suppression of apoptosis by glucocorticoids in glomerular endothelial cells: effects on proapoptotic pathways. Br J Pharmacol 2000;129(8):1673–83.
[35] Sakamoto T, Repasky WT, Chen J, Hirata A, Hirata F. Down-regulation of *bcl-xs* gene expression in rat thymocytes by dexamethasone. Biochem Biophys Res Commun 1995;215(2):511–6.
[36] Nagata S. Fas-induced apoptosis and diseases caused by its abnormality. Genes Cells 1996;1(10):873–9.
[37] Shah OJ, Anthony JC, Kimball SR, Jefferson LS. Glucocorticoids oppose translational control by leucine in skeletal muscle. Am J Physiol Endocrinol Metab 2000;279(5):E1185–90.
[38] Shah OJ, Kimball SR, Jefferson LS. Acute attenuation of translation initiation and protein synthesis by glucocorticoids in skeletal muscle. Am J Physiol Endocrinol Metab 2000;278(1):E76–82.
[39] Flusser G, Ginzburg V, Meyuhas O. Glucocorticoids induce transcription of ribosomal protein genes in rat liver. Mol Cell Endocrinol 1989;64(2):213–22.
[40] Amsterdam A, Keren-Tal I, Aharoni D, Dantes A, Sasson R, Hirsh L. Steroidogenesis and apoptosis in the mammalian ovary. Steroids 2003 (in press).
[41] Amsterdam A. Novel genes regulated by gonadotropins in granulosa cells: new perspectives on their physiological functions. Mol Cell Endocrinol 2003;17(10):1256–66.
[42] Sasson R, Dantes A, Tajima K, Amsterdam A. Novel genes modulated by FSH in normal and immortalized FSH-responsive cells: new insights into the mechanism of FSH action. FASEB J 2003;202(1/2): 133–7.
[43] Robles R, Tao XJ, Trbovich AM, Maravel DV, Nahum R, Perez GI, Tilly KI, Tilly JL. Localization, regulation and possible consequences of apoptotic protease-activating factor-1 (Apaf-1) expression in granulosa cells of the mouse ovary. Endocrinology 1999;140(6): 2641–4.
[44] Pru JK, Tilly JL. Programmed cell death in the ovary: insights and future prospects using genetic technologies. Mol Endocrinol 2001;15(6):845–53.
[45] Hosokawa K, Aharoni D, Dantes A, Shaulian E, Schere-Levy C, Atzmon R, Kotsuji F, Oren M, Vlodavsky I, Amsterdam A. Modulation of Mdm2 expression and p53-induced apoptosis in immortalized human ovarian granulosa cells. Endocrinology 1998; 139(11):4688–700.
[46] Smulson ME, Simbulan-Rosenthal CM, Boulares AH, Yakovlev A, Stoica B, Luo R, Haddad B, Wang ZQ, Pang T, Jung M, Dritschilo A, Rosenthal DS. Roles of poly(ADP-ribosyl)ation and PARP in apoptosis, DNA repair, genomic stability and functions of p53 and E2F-1. Adv Enzyme Regul 2000;40:183–215.
[47] Sasson R, Hosokawa K, Dantes A, Jung Y, Barash A, Granot I, Amsterdam A. Glucocorticoids enhance steroidogenesis and reduce apoptosis in human granulosa cells. In: Proceeding of the XIIIth Ovarian Workshop: Molecular and Cellular Basis of Paracrine, Autocrine and Juxtacrine Communication in the Ovary, Madison WI, USA, 2000.
[48] Peluso JJ, Pappalardo A, Trolice MP. N-cadherin-mediated cell contact inhibits granulosa cell apoptosis in a progesterone-independent manner. Endocrinology 1996;137(4):1196–203.
[49] Aharoni D, Meiri I, Atzmon R, Vlodavsky I, Amsterdam A. Differential effect of components of the extracellular matrix on differentiation and apoptosis. Curr Biol 1997;7(1):43–51.
[50] Foty RA, Corbett SA, Schwarzbauer JE, Steinberg MS. Dexamethasone up-regulates cadherin expression and cohesion of HT-1080 human fibrosarcoma cells. Cancer Res 1998;58(16):3586–9.
[51] Ren P, de Feijter AW, Paul DL, Ruch RJ. Enhancement of liver cell gap junction protein expression by glucocorticoids. Carcinogenesis 1994;15(9):1807–13.
[52] Gebauer G, Peter AT, Onesime D, Dhanasekaran N. Apoptosis of ovarian granulosa cells: correlation with the reduced activity of ERK-signaling module. J Cell Biochem 1999;75(4):547–54.

[53] Richards JS. New signaling pathways for hormones and cyclic adenosine 3′,5′-monophosphate action in endocrine cells. Mol Endocrinol 2001;15(2):209–18.
[54] Westfall SD, Hendry IR, Obholz KL, Rueda BR, Davis JS. Putative role of the phosphatidylinositol 3-kinase-Akt signaling pathway in the survival of granulosa cells. Endocrine 2000;12(3):315–21.
[55] Johnson AL, Bridgham JT, Swenson JA. Activation of the Akt/protein kinase B signaling pathway is associated with granulosa cell survival. Biol Reprod 2001;64(5):1566–74.
[56] Chauhan D, Pandey P, Ogata A, Teoh G, Treon S, Urashima M, Kharbanda S, Anderson KC. Dexamethasone induces apoptosis of multiple myeloma cells in a JNK/SAP kinase independent mechanism. Oncogene 1997;15(7):837–43.
[57] Singleton JR, Baker BL, Thorburn A. Dexamethasone inhibits insulin-like growth factor signaling and potentiates myoblast apoptosis. Endocrinology 2000;141(8):2945–50.
[58] Peluso JJ, Pappalardo A, Fernandez G. E-cadherin-mediated cell contact prevents apoptosis of spontaneously immortalized granulosa cells by regulating Akt kinase activity. Biol Reprod 2001;64(4):1183–90.
[59] Hillier SG, Tetsuka M. An anti-inflammatory role for glucocorticoids in the ovaries? J Reprod Immunol 1998;39(1/2):21–7.
[60] Hosokawa K, Dantes A, Schere-Levy C, Barash A, Yoshida Y, Kotsuji F, Vlodavsky I, Amsterdam A. Induction of Ad4BP/SF-1, steroidogenic acute regulatory protein, and cytochrome P450scc enzyme system expression in newly established human granulosa cell lines. Endocrinology 1998;139(11):4679–87.
[61] Michalovitz D, Halevy O, Oren M. Conditional inhibition of transformation and of cell proliferation by a temperature-sensitive mutant of p53. Cell 1990;62(4):671–80.
[62] Shastri N, Gonzalez F. Endogenous generation and presentation of the ovalbumin peptide/Kb complex to T cells. J Immunol 1993;150(7):2724–36.