The Two Faces of Janus: Sex Steroids as Mediators of Both Cell Proliferation and Cell Death

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In this issue of the Journal, Song et al. (1) report that prolonged estrogen withdrawal in breast cancer cells results in the acquisition of an apoptotic response to 17β-estradiol; i.e., the very same hormone that mediates the proliferation of its target cells in the female genital tract and mammary glands is also able to trigger their death. In this editorial, we aim to integrate these experimental results, which are obviously relevant to the management of breast cancer, within a biologic perspective.

The exploration of nature never ceases to surprise biologists. When adopting a narrow anthropocentric approach, we imagine nature as designed by a meticulous engineer. However, a more appropriate metaphor would be to imagine instead the workings of nature as the fruit of tinkering, or putting old devices to new uses. From this evolutionary perspective, it is conceivable that the same sex steroid hormones mediate the increase of cell numbers, the inhibition of cell proliferation, the inhibition of cell death, and the increase of cell death.

In the 1970s, Bruchovsky et al. (2) showed that androgens, in addition to inhibiting cell death and inducing cell proliferation in the rat prostate in situ, inhibit prostate cell proliferation. The proliferative and inhibitory effects were dissociated temporarily; i.e., the latter event followed the former. Gorski and colleagues (3,4) described an equivalent effect by estrogens for the uterus and the pituitary gland.

I N D U C T I O N O F C E L L P R O L I F E R A T I O N

For the last 20 years, the following three hypotheses have coexisted to explain how estrogens mediate the proliferation of their target cells: 1) Estrogens directly induce cell proliferation (direct-positive hypothesis), 2) estrogens mediate cell proliferation by regulating the action of putative growth factors (indirect-positive hypothesis), and 3) estrogens induce cell proliferation by canceling the effect of a plasma-borne inhibitor (indirect-negative hypothesis). We favor the third hypothesis, both because proliferation is the default state of cells in all organisms (5) and because experimental evidence shows that serum albumin inhibits the proliferation of estrogen target cells, with estrogens merely neutralizing this inhibitory effect (6,7).

I N H I B I T I O N O F C E L L P R O L I F E R A T I O N

Prolonged exposure to sex steroids results in the inhibition of cell proliferation in their target organs. It is surprising that this phenomenon of profound biologic significance and therapeutic promise has attracted the attention of few researchers, given the availability of established cell lines that express both proliferative and inhibitory responses to sex steroids (8–11). The rat pituitary GH3 cell line (12), as well as early passages of MCF-7 breast cancer cells (13), exhibited a biphasic response to estrogens: Low doses increased cell proliferation, whereas high doses inhibited cell proliferation. Similarly, human prostate carcinoma LNCP-FGC cells respond to androgens biphasically (8). Low doses of androgens increase the percentage of these cells in S phase and their proliferation rate, and high physiologic doses result in G0/G1 arrest (10). It is interesting that PC3 prostate carcinoma and MCF-7 cells stably transfected with the human androgen receptor frequently acquire the ability to respond to androgens by entering proliferative quiescence (14,15). These transfectants, along with human prostate carcinoma LNCaP cell variants that express exclusively the proliferative or the inhibitory phenotype, have provided a useful model for elucidating the molecular events that underlie these phenomena (8,10,16–18).

The inhibitory effect of androgens is mediated by the AS3 gene, which encodes a putative transcription factor (18). Studies aimed at identifying genetic factors that may be involved in prostate cancer revealed loss of heterozygosity at the AS3 locus in familial metastatic prostate cancer (19). LNCaP cells that were exposed to androgen-free medium developed into variants that proliferated maximally in this medium and responded to androgens by arresting their proliferation. This behavior was also observed when the cells were inoculated into nude mice (8,10–11). These findings suggest that the inhibitory effect of androgens on tumor growth may have the potential to be used for prostate cancer treatment (10,11).

I N D U C T I O N O F C E L L D E A T H

The E8CASS cell line was established from MCF-7 cells after long-term exposure to estrogen-free medium. This protocol was chosen to select for variants that proliferated maximally in the absence of 17β-estradiol. In addition to exhibiting the expected phenotype, E8CASS cells respond to 17β-estradiol by undergoing apoptosis (20). Song et al. (1) report that a similarly selected MCF-7 cell variant, LTED, responds to estrogens like the E8CASS cells. In addition, they cite clinical studies that show that the rate of response to diethylstilbestrol (DES) increases proportionally to the length of the postmenopausal period at the initiation of the treatment (21). Hence, they propose that long-term estrogen deprivation may play a causal role in the acquisition of this response. We interpret the generation of these two cell lines as a probabilistic, rather than a deterministic, phenomenon because several long-term estrogen-withdrawal experiments performed in our laboratory have resulted in variants that behave like BSK3, a cell line that does not express an apoptotic response to estrogens (22). Moreover, long-term es-

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trogen exposure of E8CASS cells has resulted in the selection of A2E8CASS cells, a “revertant” line with properties similar to those of MCF-7 cells—i.e., 17β-estradiol mediates their proliferation by canceling serum inhibition (20). These observations suggest that both selective conditions resulted in adaptive, epigenetic changes rather than in the selection of true genomic mutants.

The development of MCF-7 cells into a variant cell line, E8CASS, that is able to proliferate maximally in the absence of estrogens and also in the presence of charcoal–dextran-stripped serum was gradual. It involved an intermediate step in which 17β-estradiol increased the proliferation rate at concentrations lower than those required to induce apoptosis (Sonnenschein C, Soto AM: unpublished results). Similarly, LTED cells grow as tumors in nude mice at a higher rate when they are exposed to low estrogen levels and at lower rates when they are exposed to high estrogen levels (23). From the perspective of the indirect-negative hypothesis on estrogen induction of cell proliferation, the partial loss of estrogen-mediated cell proliferation is probably the consequence of a loss of sensitivity to the serum-borne inhibitor albumin (7).

As Song et al. (1) pointed out, the acquisition of an apoptotic response to 17β-estradiol by MCF-7 cells upon long-term estrogen withdrawal parallels the effect of androgen deprivation on LNCaP cells. However, unlike E8CASS cells, the resulting LNCaP cell variant (i.e., the MOP cell line) responds to high androgen levels by entering quiescence, a response present in the parental cell line (24). This behavior is maintained when MOP cells are inoculated into nude mice (24). These discrete LNCaP phenotypes—i.e., androgen inhibition of cell proliferation and androgen induction of cell death—provide the basis to support the administration of androgens to prostate cancer patients, once reliable markers for the inhibitory and apoptotic response are found. These data also provide additional support for intermittent androgen-withdrawal treatment, which was originally proposed to avoid the selection of androgen-resistant phenotypes (25).

Unlike the findings in the LNCaP model, the estrogen-induced apoptotic response of the MCF-7 variants seems to coexist with a reduced but significant proliferative effect as demonstrated in both LTED and E8CASS (Soto AM, Sonnenschein C: unpublished data) cells. Another difference between the androgen and estrogen models is that cells stably transfected with estrogen receptor-α usually express estrogen-induced apoptosis (26), whereas cells stably transfected with the androgen receptor express androgen inhibition of cell proliferation (14, 15). Estrogen-induced apoptotic responses have also been reported in normal neurons (27). It is noteworthy, however, that the induction of apoptosis by these hormones has not been observed in normal male and female genital tracts and mammary glands but has been described in cancer cells maintained in culture and in carcinomas derived from these organs in vivo.

What mechanisms underlie the induction of apoptosis by estrogens? Song et al. (1) hypothesize that this effect is mediated by the Fas/Fas ligand (FasL) pathway. When they compared the expression of Fas and FasL in cells that showed estrogen-induced apoptosis and in cells that were devoid of this response, they found that estrogens induced FasL both in LTED and in the parental MCF-7 cells. LTED and E8CASS cells express Fas proteins constitutively, whereas MCF-7 cells do not. These data are consistent with, but do not prove, that LTED and E8CASS undergo apoptosis because they express Fas and that MCF-7 and BSK3 cells do not undergo apoptosis because they do not express Fas. An alternative mechanism was proposed by Szelenyi et al. (28), who used subtracted libraries to identify sequences expressed exclusively in E8CASS cells during estradiol induction of apoptosis. One candidate complementary DNA, E9, showed homology with Requiem, a mediator of apoptosis (29). Thus, the hypotheses that 17β-estradiol mediates this response by inducing E9, FasL, or both require further testing.

ESTROGEN-INDUCED CELL DEATH IN TREATMENT OF BREAST CANCER

Hormone additive therapy (DES) was effective in the treatment of breast cancer before the advent of antiestrogens (30). Tamoxifen treatment superseded the use of DES because the former was better tolerated than the latter (31). However, tumors that ceased to respond to tamoxifen underwent clinical regression after DES treatment (32), suggesting different underlying mechanisms. As Song et al. (1) mentioned, a recent update of the original trial comparing DES with tamoxifen demonstrated that patients treated with DES survived longer than patients receiving tamoxifen. It is also noteworthy that the success of this treatment increased with the length of menopause at the start of DES therapy, suggesting that the positive response is more likely to occur after prolonged estrogen withdrawal. Song et al. (1) propose that these findings “emphasize the need to reconsider use of additive estrogen therapy in breast cancer patients.” However, since the response rate at its best is 22%, it would be desirable to develop markers of response so that only selected candidates would be exposed to estrogens. This cautious strategy is based on the argument that these hormones may still increase the tumor mass by favoring the proliferation of estrogen receptor-positive cells that do not express the apoptotic response.

Finally, we concur with Song et al. (1) that sex steroid-induced apoptosis merits continued investigation. Furthermore, in addition to testing the hypothesis that the regression of clinical cancer by additive hormone therapy is due to direct hormone action on the epithelial cells, the role that reciprocal stroma–epithelium interactions play in estrogen-induced regression should also be explored (33). A better understanding of sex steroid-induced apoptosis, a biologic phenomenon that seems to be highly context dependent, may ultimately improve the prognosis of breast and prostate cancer patients alike.

REFERENCES


