Molecular Mechanism for Breast Cancer Incidence in the Women's Health Initiative

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ABSTRACT

The Women's Health Initiative (WHI) was designed to evaluate the benefits of hormone replacement therapy. The primary goal was to establish the value of synthetic progestin and estrogen or estrogen alone to reduce the risk of coronary heart disease (CHD). The estrogen/synthetic progestin trial was stopped at 5.2 years and the estrogen trial was stopped after 6.8 years. Although the estrogen/synthetic progestin trial was stopped for the anticipated rise in the risk of breast cancer, the estrogen trial was stopped for elevation of strokes. Women taking estrogen/synthetic progestin or estrogen alone had no benefit from a reduction in CHD. Paradoxically, there was a decrease in breast cancer incidence in the estrogen trial. The decrease in breast cancer was sustained. The elevation of breast cancer in the estrogen/synthetic progestin trial was also sustained a decade after stopping treatment. Evidence is presented to explain the paradoxical sustained decrease in breast cancer with estrogen and the mechanism for the reversal of breast cancer incidence and mortality with the mixed synthetic progestin/glucocorticoid actions of the synthetic progestin used with estrogen in women with an intact uterus. The fact that the WHI study had an estrogen deprivation gap of at least 5 years, introduced an experimental biological dimension not observed in medical practice using progestin/estrogen hormone replacement. The evidence presented confirms the known human cancer biology of estrogen action.

Introduction

Clinical trials and clinical observations create databases that permit advances in healthcare. If a question is asked, but the reply does not comply with your working model of "what should be the correct answer," your model is incorrect. So it is with the interpretation of the results from the Women's Health Initiative (WHI). Sex steroids conjugated equine estrogen (estrogen) plus medroxyprogesterone acetate (synthetic progestin; refs. 1, 2) cause an increase in the incidence and mortality for breast cancer. We know that happens, because that is what sex hormones do (1). However, the paradox (2), which is maintained throughout the WHI evaluation of more than 12 years, is estrogen causes a decrease in mortality and a decrease in the incidence of new breast cancers. This is counter intuitive to the scientific and medical community unless one embraces and understands the known clinical evidence that governs safe estrogen use for the treatment of breast cancer after menopause (3, 4). These were established 70 years ago.

An estrogen deprivation gap of 5 years after menopause is required for high-dose estrogen to be an effective treatment for breast cancer (**Table 1**; ref. 3). In addition, the same applies to 5 years of adjuvant tamoxifen therapy when recurrence and mortality continue to decrease after adjuvant tamoxifen treatment is stopped (5, 6). In this case, the patient and her micrometastasis have received 5 years of an "antiestrogen-induced estrogen deprivation gap" to prevent the growth of estrogen-dependent micrometastases. Once tamoxifen is cleared from the body and estrogen again bathes micrometastases, micrometastatic breast cancer growth does not occur (5). Laboratory findings provided an explanation (6). Here, the focus will be the results of WHI trial, but the evolution of drug resistance with tamoxifen in breast cancer is instructive and will be integrated as a confirmatory model of translation research to aid patient survival.

The use of either estrogen in postmenopausal women without a uterus or estrogen plus a synthetic progestin for women with an intact uterus, continues to be controversial despite 70 years of trial and error. Initially, estrogen was used by all postmenopausal women, but reports of an increase in endometrial cancer (7, 8) resulted in the FDA approval of estrogen plus synthetic progestin for women with an intact uterus. The synthetic progestin prevented the development of endometrial cancer. The principal uses of hormone replacement therapy (HRT) were to control menopausal symptoms and for the treatment of osteoporosis that results after menopause.

However, evidence from nonrandomized clinical studies and epidemiology supported the position that HRT was beneficial for the prevention of heart disease. The incidence of coronary heart disease (CHD) rises significantly a decade after menopause. In addition, no major randomized clinical trials have addressed the overall health benefits of HRT. As a result the WHI was planned as two trials to establish definitively the

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Table 1. Objective response rates in postmenopausal women with metastatic breast cancer using high-dose estrogen therapy.

Age since menopause	Numbers of patients	Percentage responding
Postmenopausal		
0–5 years	63	9
>5 years	344	35

Note: A total of 407 patients were classified on the basis of the time from menopause (16).

value of estrogen or estrogen plus a synthetic progestin in randomized placebo-controlled study populations, the majority of whom were 10 years past the menopause.

Summary of the WHI: Estrogen Plus Synthetic Progestin Trial

A total 16,608 postmenopausal women with an intact uterus were randomized to either placebo (8,102) or estrogen plus synthetic progestin (8,506). The age distribution was 50–59 years, estrogen plus progestin (2,839) and placebo (2,683); 60–69 years, estrogen plus progestin (3,854) and placebo (3,657); and 70–79 years, estrogen plus progestin (1,814) and placebo (1,762). The trial was stopped after a mean of 5.2 years of follow-up. Overall, the health risks, that is, CHD, breast cancer, stroke, and pulmonary emboli, exceeded benefits and did not support the use of estrogen plus a synthetic progestin for the primary prevention of CHD in women with an intact uterus (1).

Summary of WHI/Estrogen Trial

A total of 10,739 postmenopausal hysterectomized women were randomized to either placebo (5,429) or estrogen (5,310). The age distribution of women was 50–59 years, estrogen (1,673) and placebo (1,639); 60–69 years, estrogen (2,387) and placebo (2,465); and 70–79 years, estrogen (1,286) and placebo (1,281). The trial was stopped after an average of 6.8 years. Overall, the burden of disease was the same between estrogen and placebo, suggesting no overall benefit. Estrogen could not be recommended for disease prevention (2).

Unexpectedly, a possible reduction of invasive breast cancer was noted: placebo 124 and estrogen 94, and this observation merited a follow-up analysis (9). The authors concluded that after a median of 11.8 years of follow-up and a median of 5.9 years of estrogen alone, there remained a lower incidence of breast cancer (placebo 199 cases and 151 estrogen cases). More importantly, risk reduction was noted in women without risk factors ($P = 0.02$) or benign breast disease ($P = 0.01$), and fewer women died of breast cancer [HR, 0.37, 95% confidence interval (CI), 0.13–0.91; $P = 0.03$] in the estrogen group, six deaths per year, compared with the placebo group (16 death per year).

Subsequently, other publications have followed and documented the WHI trial (10, 11).

Recently, Chlebowski and colleagues presented a final analysis of the breast cancer incidence in the WHI trials (12). While receiving estrogen alone there was a significantly lower rate of breast cancer compared with placebo (HR, 0.76; 95% CI, 0.58–0.98; $P = 0.04$). In contrast, the estrogen plus synthetic progestin trial had a significantly higher rate of breast cancer compared with the placebo group (HR, 1.26; 95% CI, 1.02–1.56; $P = 0.04$).

A decade later, after discontinuing estrogen plus progestin, breast cancer incidence increased (HR, 1.29; 95% CI, 1.14–1.47; $P < 0.001$). These women had a 45% higher risk of dying from breast cancer (HR, 1.45; 95% CI, 0.98–2.015; $P = 0.06$). In addition, there was a 29% higher risk of dying after breast cancer diagnosis (HR, 1.29; 95% CI, 1.02–1.63; $P = 0.03$).

In contrast, the WHI estrogen trial, a decade after discontinuing treatment, had a significantly lower rate of breast cancer (HR, 0.56; 95% CI, 0.34–0.92; $P = 0.02$) and both a lower risk of dying from breast cancer (HR, 0.56; 95% CI, 0.34–0.92; $P = 0.02$) or after breast cancer diagnosis (HR, 0.75; 95% CI, 0.56–1.02; $P = 0.06$).

A sustained beneficial antibreast cancer action of estrogen alone noted in the WHI study is counter intuitive because the dogma is that estrogen, through the estrogen receptor (ER), is the primary signal for the initiation and growth of breast cancer. This fact is reinforced by reference to an American Society of Clinical Oncology (ASCO) evaluation. The committee states: *"the development of therapeutics for ER-expressing breast cancer has been one of the great clinical advances of the past 50 years and has served as a paradigm for the development of targeted therapies in oncology." "As most breast cancers are ER positive and given the world wide prevalence of the disease, it is arguable that antiestrogen treatments have had greater global impact than any other treatment intervention in cancer medicine."* (13).

Hypothesis

The hypothesis to be addressed is that the sustained decrease in breast cancer in the WHI estrogen trial results from the apoptotic actions of low-dose estrogen following a long-term estrogen deprivation gap and this biology is reversed by the glucocorticoid action of the synthetic progestin in the synthetic progestin/estrogen trial.

The questions to be addressed:

(i) Under what clinical circumstances does estrogen change from being the fuel to trigger the growth of breast cancer to becoming the killer of breast cancer cells?

(ii) If estrogen does kill breast cancer cells in patients, why does the synthetic progestin prevent estrogen-induced breast cancer cell death and increase breast cancer incidence?

To answer the first question, we must return to the origins of chemical therapy (chemotherapy) in the 1940s.

A Chemical Treatment for Cancer

Professor Alexander Haddow created the first successful chemical therapy to treat select cancers (14). Haddow used animal models of cancer and paradoxically found that polycyclic carcinogenic chemicals could retard the growth of some tumors. During this period, in the 1930s, there was an explosion in the structure–function relationships of synthetic estrogens and they too had multiple benzene rings (15). His translational research resulted in the discovery that only breast and prostate cancer responded to high-dose estrogen therapy; no other human cancers were responsive (14).

However, there was a rule that had to be obeyed. The success of estrogen therapy in breast cancer was dependent on the time from menopause in postmenopausal women with stage IV breast cancer. Haddow and David (16) stated:

> *"when the various reports were assembled at the end of that time, it was fascinating to discover that rather general impression, not sufficiently strong from the relatively small numbers in a single group, became reinforced to the point of certainty; namely, the beneficial responses were three times more frequent in women over the age of 60 than in those under that age; that on the contrary accelerate the course of mammary cancer in younger women and that their therapeutic use should be restricted to cases five years beyond menopause. Here was an early and satisfying example of the advantages which may accrue from cooperative clinical trials."*

A database from Stoll (17) is illustrated in **Table 1**. An estrogen deprivation pause of at least 5 years following menopause was essential to obtain an optimal antibreast cancer effect for a synthetic estrogen. High-dose estrogen therapy became the standard of care for metastatic breast cancer until the development of the antiestrogen tamoxifen was initiated in the early 1970s (18). Tamoxifen went on to be used to treat all stages of ER-positive breast cancer as well as chemoprevention.

The rationale to switch from high-dose estrogen therapy to tamoxifen for the treatment of metastatic breast cancer was not increased efficacy, but the fact that tamoxifen had fewer side effects (19, 20). However, the rationale and mechanism of action of tamoxifen for the treatment of breast cancer was clear in 1981, that is, tamoxifen blocks the action of estrogen that stimulates tumor growth by binding to the tumor ER. The ER is deactivated once bound to the nonsteroidal antiestrogen tamoxifen or its metabolites. In contrast, the mechanism of the antitumor action of high-dose estrogen was unknown. This was summarized by Haddow and David (16) in the inaugural ASCO/Karnofsky Memorial Lecture in 1970. *"The extraordinary extent of tumor regressions observed in perhaps 1% of postmenopausal cases (with estrogen) has always been regarded as of major theoretical importance, and it is a matter for some disappointment that so much of the underlying mechanisms continue to allude us."*

It is important to observe that an early randomized trial of tamoxifen versus diethylstilbestrol (20) was reanalyzed (21) and a survival benefit for diethylstilbestrol was noted over tamoxifen. Clearly, the antitumor mechanism of diethylstilbestrol was of scientific interest. Therefore, it is perhaps ironic that the first understanding of the antitumor effects of estrogen (22, 23) would come from an understanding of acquired drug resistance to tamoxifen, but this was not a preplanned research strategy. The accident was a discovery. Unanticipated laboratory discoveries energize research advances. Discovery illuminated a path to solve a clinical paradox. This is that story.

The Evolution of Tamoxifen Resistance in Breast Cancer Cells *In Vivo*

The first understanding of the complexity of tamoxifen resistance *in vivo* was made using the remarkable ER-positive MCF7 breast cancer cells (24) transplanted into ovariectomized athymic immunodeficient mice (25). Estrogen-stimulated tumor growth was blocked, but eventually tumor growth occurred despite continuing tamoxifen treatment (26). However, retransplantation of tumors into new generations of ovariectomized athymic mice demonstrated that tumors actually depended on either tamoxifen or estrogen to grow and were not exhibiting hormone-independent growth (27, 28). This was the first demonstration of a human cancer that is dependent upon the anticancer therapy to grow. Ultimately, these new published concepts and the testing of a new pure steroidal antiestrogen (29) *in vivo*, were essential first steps to plan the clinical trials, a decade later, which showed that either an aromatase inhibitor (i.e., no estrogen to stimulate the tumor in athymic mice after tamoxifen is stopped) or fulvestrant could be a useful second-line therapy for patients resistant to first-line tamoxifen (30, 31).

The problem in the laboratory, which fortunately was not addressed, was that the tamoxifen-stimulated MCF7 tumor cells could not be grown successfully in culture. As a result, it was decided to continue to passage the MCF7 tumor-associated macrophage (TAM) tumors for years *in vivo*, never considering the possibility that tamoxifen resistance could evolve. This it did over 5 years, but herein lies a tale.

After Marco Gottardis had finished his PhD, with four peer-reviewed publication that changed clinical care (27, 29, 32, 33), Doug Wolf, another PhD student, was given the project to use Marco's MCF7 TAM tumors *in vivo* to determine the growth factors responsible for either estrogen- or tamoxifen-stimulated tumor growth. All went well until Doug discovered that MCF7 TAM tumors, now some 5 years after Marco's studies, did not grow with physiologic estrogen treatment, but immediately regressed (22). He repeated the experiments several times but now recommended that we contact the editor of *Cancer Research* to withdraw Marco's articles, as they could not be reproduced. However, Haddow was speaking to us, and it was a discovery. I presented the results at the St. Gallen Breast Cancer meeting (22) stating *"that the prolonged antitumor effects of tamoxifen continuing after 5 years of adjuvant therapy is a direct result of the cytocidal effects of estrogen." "We should not give*

tamoxifen forever, as the action of stopping long-term adjuvant tamoxifen to expose the tumor to the patient's own estrogen provides the survival benefit." Our data, once reproduced and published in the referred literature (23), presented the first studies of the antitumor effects of estrogen that either:

(i) provide a long-term survival action for tamoxifen after 5 years of adjuvant tamoxifen therapy (6) or
(ii) low-dose estrogen could be used as a salvaged therapy following the failure of tamoxifen in the treatment of metastatic breast cancer. Ellis and colleagues (34) successfully addressed this published proposal, following the development of aromatase inhibitor drug resistance in patients with breast cancer.

In the 2000s the scene was now set for the development of new models *in vitro* to study the mechanism of action of estrogen as an antibreast cancer agent in long-term estrogen-deprived (LTED) breast cancer that would be resistant to aromatase inhibitor therapy.

Breast Cancer Models to Decipher Mechanisms of Estrogen-induced Apoptosis

Song and colleagues studied the influence of LTED on the growth and actions of high-dose estrogen treatment on the MCF7 breast cancer cells *in vitro* (35). The group was particularly interested in the antitumor mechanism of high-dose estrogen to solve the therapeutic paradox noted by Haddow and colleagues (16). Nevertheless, they also completed concentration–response curves so their data also applied to low-dose estrogen therapy (36). Apoptosis was detected and a mechanism was proposed through the cell membrane Fas/FasL system (35). Studies *in vivo* using either tamoxifen-stimulated MCF7 tumors (37) or raloxifene-stimulated MCF7 tumors (38), both concluded that estrogen decreased NF-κB and HER2/Neu, and increased Fas expression in selective ER modulator (SERM)-stimulated tumors. Studies *in vivo* with raloxifene-stimulated MCF7 cells illustrated the interconversion of estrogen- and SERM-stimulated growth over a decade (39). In contrast, Lewis and colleagues (40) discovered that cloned MCF7 5C cells *in vitro*, derived from LTED MCF7 cells (41), underwent apoptosis only under the correct serum conditions. Using this specific MCF7 5C cell model, they discovered (42) that the mitochondrial pathway of apoptosis has a primary role in estrogen-induced apoptosis followed by the activation of death receptor pathways (Fas/Fas ligand) for cellular execution (see **Fig. 1**).

To decipher the time-dependent initiation mechanism of estrogen-induced apoptosis, Ariazi and colleagues (43) compared and contrasted a time course of gene array analyses over 7 days. They used a cloned MCF7 WS8 (wild-type), MCF7 5C LTED clone and MCF7 2A another LTED clone (44–46). Biologically, MCF7 2A cells are of interest as they were originally classified as estrogen unresponsive for growth or apoptosis. Subsequently, the cells were found to require 2 weeks to trigger partial apoptotic cell death (43).

Ariazi and colleagues (43) created a methodology called differential AUC analysis to identify genes uniquely regulated in MCF7 5C cells and, therefore, were associated with estrogen-induced apoptosis. Both inflammatory response genes and endoplasmic reticular stress (ERS) were overrepresented in MCF7 5C cells. In fact, the ERS genes suggested E_2 inhibited protein folding, translation, and fatty acid synthesis. Furthermore, ERS-associated apoptotic genes *Bcl-2* and *Caspase 4* mediated cell death. Overall, the increase in inflammatory genes suggested a negative role for anti-inflammatory agents that might prevent estrogen-induced apoptosis.

The plasticity of LTED breast cancer cells to change to different forms of resistance to SERMs or LTED in the laboratory provided the framework for understanding the strategies to use new therapies for treatment in the clinic. The ubiquitous *c-Src* gene in breast cancer was found to be important to modulate shifts in drug resistance and apoptosis in LTED cells treated with estrogen (47–51). This observation may yet prove to be a clue to future drug development.

An alternative way of interrogating LTED breast cancer cells during ER-mediated apoptosis is to consider the structure–function relationships of ER-binding ligands that will modulate apoptotic cell death (52–55). In this way, small alterations of the shape of the estrogen–ER complex can be predictably correlated with apoptotic cell death. Through structure–function relationships, it has been possible to identify the specific amino acid, THR 347 that must be displaced in the ER by a phenolic hydroxyl to delay the unfolded protein response (UPR) and apoptosis (56). Relevant to this discussion, is the apoptotic potential of the constituents of conjugated equine estrogen (CEE) (57). The main constituents: estrone, equilin, and equilenin all trigger apoptosis in LTED breast cancer cells.

Not only does the molecular pharmacology of novel ligands that bind to the ER predictably modulate estrogen-induced apoptosis (58–61), but also justifies the examination of therapeutic agents to be used for the treatment of metastatic breast cancer that has become resistant to aromatase inhibitors. The goal is to trigger estrogen-induced apoptosis by using "weak" estrogens that do not have the high potency side effects of estradiol. Two candidates are in clinical trial: estetrol (62, 63) and Selective Human ER Partial Agonists (64, 65).

Recently, Hosford and colleagues (66) have addressed the mechanism of estrogen-induced apoptosis using a panel of ER^+ models that were resistant to LTED. Models included LTED, MCF7 cells, murine mammary carcinoma, C4-HI, C7-2-HI, and a patient-derived xenograph. It was concluded that estrogen induction of apoptosis depends upon JNK signaling, p53, and an UPR with an amplification of ESR1. These comparative studies of cells from culture and patient-derived xenographs integrate the mechanisms into a confirmatory matrix of models.

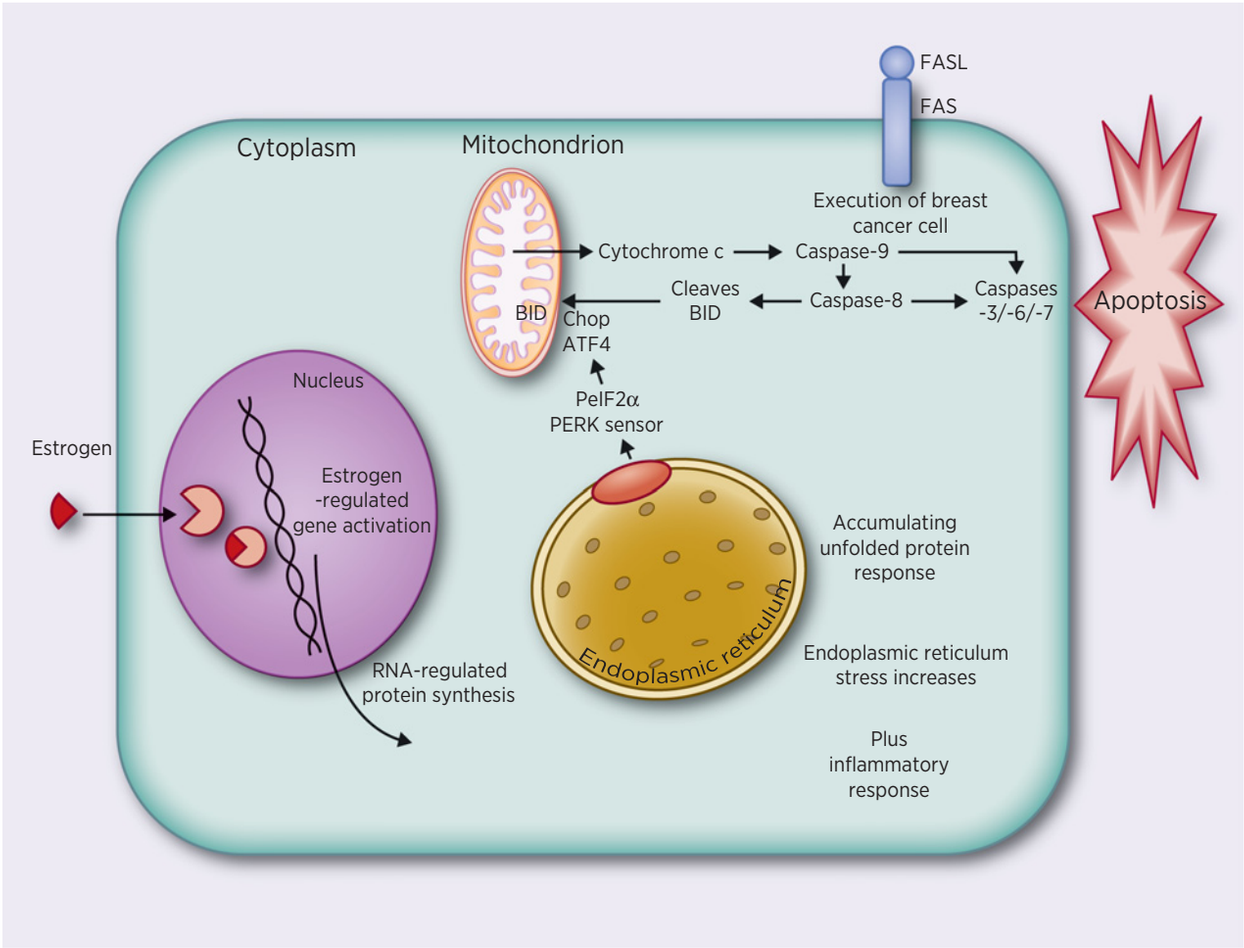

Figure 1.

Under normal circumstances, the ER-responsive breast cancer binds estrogen to increase replication of the cell population. In contrast, during long-term (5 years) estrogen deprivation following menopause, during aromatase inhibitors or SERMs treatment, the breast cancer cell survival mechanisms are reconfigured to favor estrogen-independent growth. Estrogen now binds to the nuclear ER to activate gene-specific mRNA synthesis in the endoplasmic reticulum. However, this overproduction of new proteins creates an UPR that is monitored by the PERK sensor to elevate eukaryotic initiating factor 2 alpha. This event blocks global protein translation. However, the preferential high expression of proteins, for example, activating transcription factor 4 (ATF4) and C/EBP homologous protein enables apoptosis (75). It has been reported (42) that there is an increase in the proapoptotic B-cell lymphoma 2 (BCL-2) proteins (BAX, BAK, and BIM) that in turn disrupt the mitochondrial membrane to allow the translocation of cytochrome C out of the organelle with caspase 9 activation and PARP cleavage. Further experimental details are reported in (42). Global gene expression across time has identified stress responses and massive increases in inflammatory responses to be the trigger for estrogen-induced apoptosis (43). The NF-κB noncanonical pathway was suggested (87) to be essential for cell growth that is closed down by estrogen. This was proven subsequently (74). Finally, cell execution occurs through the FAS/FASL extrinsic pathway (35, 37, 38).

Using the LTED MCF7 cell model, the second question can now be addressed.

(ii) Estrogen kills LTED breast cancer cells in patients, so why does the synthetic progestin medroxyprogesterone acetate (MPA) in the WHI prevented estrogen-induced breast cancer cell death and increased breast cancer tumor growth?

The synthetic progestins do not have specificity for the progesterone receptor alone; the molecules interact with other members of the steroid receptor super family. For example, synthetic 19 nor-testosterone derivatives that are progestins or the antiprogestin/antiglucocorticoid, RU486, is documented to have estrogenic activity and causes the growth of breast cancer cells in culture (67–71). This raises the question of whether MPA has other interactions with steroid hormone receptors? Historically, MPA is used in high doses to treat breast cancer, but one of the significant side effects is weight gain. This is a glucocorticoid effect. This clinical knowledge dovetails with the report by Ariazi and colleagues (43) that the inflammatory responses initiated during estrogen-induced apoptosis could potentially be blocked by the anti-inflammatory actions of glucocorticoids in LTED breast cancer cells.

Studies in the laboratory (72) demonstrated that MPA has glucocorticoid activity and this synthetic progestin is able to

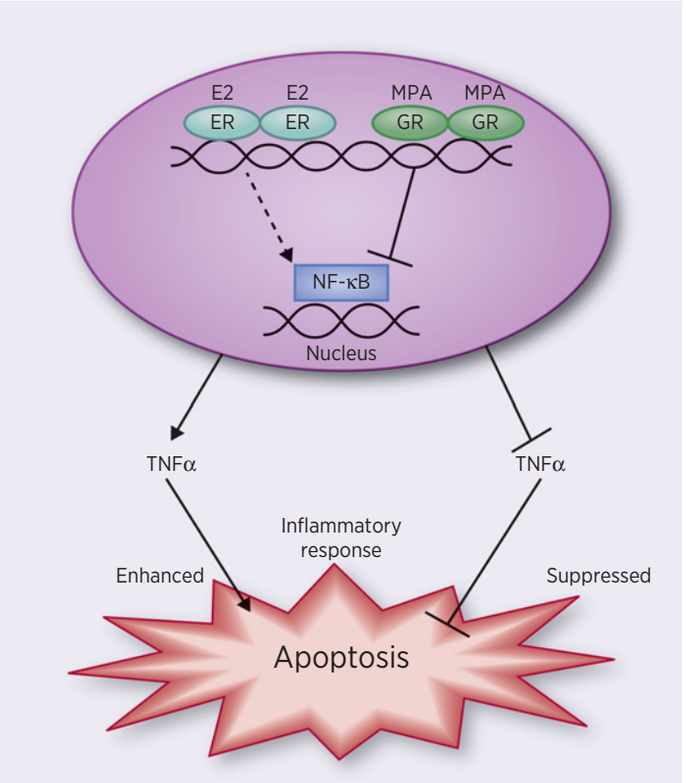

Figure 2.
Estrogen, through PERK, activates lipid metabolism–associated transcription factor CCAAT/enhancer-binding protein beta (c/EBP beta), which is responsible for suppressing NF-κB in LTED MCF7-5C cells. However, NF-κB binding activity increases when E2 treatment is prolonged. The mechanism is to increase STAT3. This enhancement of stress responses results in the release of NF-κB–dependent TNFα. This stress and inflammation response can be blocked by glucocorticoids. MPA (synthetic progestin) is not a pure progestin, but has significant glucocorticoid activity (72). The synthetic progestin, dexamethasone, through the glucocorticoid receptor, prevents stress responses and inflammation by blocking NF-κB DNA-binding activity with a blockade of TNFα production (76). This process blocks apoptosis and breast cancer cells grow.

block estrogen-induced apoptosis in LTED breast cancer cells. The pharmacology is through the glucocorticoid receptor and is blocked by RU486, which has antiglucocorticoid activity. A comparator synthetic progestin, 19-norethindrone acetate, acts as an estrogen at high doses, upregulating ER target genes and generating apoptosis. It is concluded that MPA acting as a glucocorticoid, blunts estrogen-induced apoptosis thereby increasing the risk of breast cancer growth (72).

In fact, the laboratory science has been further expanded to define the final molecular events that cause estrogen-induced apoptosis (73). A stress sensor of UPR called protein kinase RNA-like endoplasmic reticulum kinase (PERK) has an essential role in the activation of NF-κB by estrogen. Inhibition of PERK activity completely blocks the binding of STAT3 and NF-κB to DNA thereby preventing estrogen-induced apoptosis (74). Indeed, estrogen-induced apoptosis is phenocopied by blocking dephosphorylation of eukaryotic initiation factor 2 alpha (75). Further proof has recently been published that the glucocorticoid dexamethasone and MPA, the synthetic progestin, with glucocorticoid activity suppress NF-κB by binding to the glucocorticoid receptor to block estrogen-induced apoptosis (76) (see **Fig. 2**).

Thus, in summary, the precise molecular mechanism of estrogen-induced apoptosis in LTED breast cancer has been deciphered and the molecular modulation of inflammatory responses by glucocorticoids identified. However, it is the translation to clinical care, based on biological evidence that enhances progress in medicine.

Clinical Evidence that Govern the Paradoxical Actions of Estrogen alone or Combined with a Synthetic Progestin in the WHI

(i) The WHI is a major clinical trial primarily conceived to study whether estrogen alone or estrogen plus a synthetic progestin could reduce the risk of CHD. The WHI trial was designed to recruit women 5–10 years post-menopause, that is, a time when there was known to be a significant increase in CHD. The median age of both treatment trials was over the age of 60. This treatment strategy is not the usual application of estrogen/estrogen plus synthetic progestin used by women passing through the menopause; an estrogen deprivation gap was introduced into the WHI symptoms, that is, the clinical trial design was unique compared with the standard-of-care hormone replacement for the control of menopausal symptoms.

(ii) The design of WHI with an estrogen deprivation gap, created LTED and the results of the estrogen study alone were consistent with the 5-year estrogen deprivation gap that was standard of care (1950–76) for the use of high-dose estrogen to treat metastatic breast cancer before the advent of tamoxifen (**Table 1**). This, in turn, facilitates estrogen-induced apoptosis and tumor regression. However, the microscopic tumor burden in the WHI facilitates the complete eradication of early small clusters of tumor cells.

(iii) Long-term adjuvant tamoxifen therapy LTED creates the same biological situation as for micrometastatic breast cancer during adjuvant treatment. It is, therefore, of no surprise that adjuvant therapy with tamoxifen is optimal between 5–10 years of treatment. In fact, it has been demonstrated (5) that the competitive inhibitor of estrogen action, tamoxifen, continues to cause a decrease in ER disease recurrence and a decrease in mortality after adjuvant therapy stops. This is a unique clinical observation (5). It is a rule of pharmacology that a competitive inhibitor of estrogen action at the tumor ER fails to act as an anticancer agent when the patient stops taking the drug. It would be predicted that micrometastases would recur, as the ER is reactivated with circulating estrogen, but it does not. In reality, benefit for the patient continues after stopping adjuvant tamoxifen at 5 year (6).

(iv) After 5 years of tamoxifen there is no rebound of tumor growth and an increase in mortality. This is a recapitulation of the evolution of tamoxifen resistance in patients previously described in the laboratory (23). The dimension of time (5 years) of an estrogen deprivation gap is essential for tamoxifen to create ER-positive breast cancer cells that are sensitive to the apoptotic action of a woman's own estrogen (6).

(v) The HRT in the WHI does not comply with European data (77, 78), which is not clinical trial but multiple combined observational studies. All progestins used have the same carcinogenic effect on breast cancer and this amplifies the small estrogen replacement therapy (ERT) effect. This result is consistent with the fact that no gap is given in clinical practice (and hence not observed in observational studies) following menopause and LTED does not occur. Breast cancer continues to grow with administered HRT at menopause.

(vi) The WHI relied entirely on the use of a single synthetic progestin, MPA, to prevent endometrial carcinoma and protect the intact uterus. An estrogen deprivation gap of 10 years was employed. This chance choice of selecting a synthetic progestin (MPA in the United States) with known glucocorticoid activity in the WHI has the potential to block estrogen-induced apoptosis and cause breast tumor growth. However, all progestins used in the epidemiology reports (77, 78) enhanced breast cancer risk above estrogen alone. The breast did not respond to block carcinogenesis as occurs in the uterus to prevent endometrial cancer. Estrogen plus any synthetic progestin drives breast carcinogenesis and growth in cells that have no estrogen deprivation gap.

(vii) On the basis of (vi), an alternate HRT that has no other hormonal activity is required. The target tissues of uterus and breast do not respond uniformly. A progestational agent blocks endometrial carcinogenesis, but not breast carcinogenesis. One available alternative is the CEE/bazedoxifene combination medication for the amelioration of menopausal symptoms (79). The application of an SERM (80) with CEE, is an alternative HRT immediately available.

Future Considerations

The WHI provides a wealth of valuable biological data despite design shortcomings that diverge from the applications of estrogen plus a synthetic progestin or estrogen to ameliorate menopausal symptoms around 50 years of age. The goal was to assess the benefits of estrogen or estrogen plus synthetic progestin on CHD, so a 10-year estrogen deprivation gap was introduced to advance the mean age of participants to 60 years old, that is, 10 years after menopause. As a result, the rules for the use of therapeutic estrogen to treat breast cancer following an estrogen deprivation gap (**Table 1**) are replicated with the estrogen trial: there is a lower incidence of breast cancer and fewer women died of breast cancer.

Importantly, it seems that estrogen has a sustained effect to prevent breast cancer (cures?) and there are statistically fewer deaths than the placebo control group of women with no uterus. In contrast, in clinical practice with estrogen plus synthetic progestin or estrogen alone, the immediate continuation of a hormonal environment stimulates the early breast tumor cells to continue to grow. Be that as it may, less than 5 years of estrogen alone has a minimal effect on breast cancer development and that effect is not long lasting in European studies (77, 78), with no gap in clinical practice.

There are numerous clinical trials with the SERMs tamoxifen (81–83) or tamoxifen versus raloxifene (84, 85) that were evaluated in high-risk women to prevent breast cancer. Survival advantages have not been noted, although a recent study (86) using the Surveillance Epidemiology and End Results databases does provide tantalizing evidence for survival with raloxifene use.

Although medicine has the SERMs to switch-on or switch-off estrogen target sites around the body to prevent major diseases in women (80), estrogen may be viewed as an SERM of ER-positive breast cancer growth and death: physiologic estrogen stimulates breast cancer growth, but when breast cancer is starved of estrogen, under LTED conditions, remaining breast cancer cells are killed, if estrogen returns apoptotic death is triggered. Surviving cells will continue to regrow with estrogen. Breast cancer (ER^+) cell populations adapt to any environment to ensure the survival of the population.

What is most interesting is that the estrogen-induced apoptosis caused by estrogen has a sustained benefit in the estrogen WHI trial, even when a woman's own estrogen returns (cures?). There are no remaining breast cancer cells to be reactivated. Perhaps, this hypothesis can be supported with evidence of actual estrogen levels if blood draws have been taken during the WHI trial.

The estrogen trial provided an answer for breast cancer incidence and mortality that was unanticipated and is counter

intuitive. The answer from the trial is that risk reduction was noted in LTED women without either breast cancer risk factors or benign disease, and fewer women died of breast cancer in the ERT group. The question for the future is "why is that population different?" or is it the fact that risk factors have a negative effect upon the genesis of breast cancer cells that are programmed to undergo estrogen-induced apoptosis. All previous prevention trials using tamoxifen (81–83) or tamoxifen against raloxifene (84, 85) to block estrogen-stimulated tumors were selected for study because of their high level of risk factors. In contrast, estrogen use that triggers estrogen-induced apoptosis in women was noted to be without risk factors. Research efforts to understand and trigger a targeted commitment to apoptosis therapeutically, may result in new agents to kill any cancers cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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