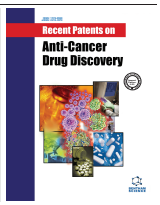
Recent Patents on Anti-Cancer Drug Discovery, 2016, *11*, 254-266

Causal Therapy of Breast Cancer Irrelevant of Age, Tumor Stage and ER-Status: Stimulation of Estrogen Signaling Coupled With Breast Conserving Surgery



Zsuzsanna Suba*

National Institute of Oncology, Surgical and Molecular Tumor Pathology Centre, Address: H-1122, Ráth György Str. 7-9, Budapest, Hungary

Zsuzsanna Suba

Received: October 13, 2015; Accepted: February 2, 2016; Revised: March 8, 2016

Abstract: ***Background:*** Results of long-term studies justify that the rate of breast cancer recurrence and tumor-related mortality remains quite unpredictable, regardless of the use of any current therapeutic measures.

Objective: Since the application of standard therapies, such as surgery, radiation, chemotherapy and antiestrogen administration does not work as might be expected; our therapeutic practice requires thorough rethinking.

Method: Published long-term therapeutic results on breast cancer cases were analyzed in correlation with stage at diagnosis, ER-status of tumors and patients' age. The effectiveness of current therapeutic measures was also compared by estimating the rate of tumor-free survival, breast cancer recurrence and breast cancer-specific mortality.

Results: Diagnosis and treatment of breast cancer at an early stage cannot improve the rate of tumor-free survival. Poor differentiation of tumors, ER-negativity in particular, defines poor prognosis even after applying aggressive therapies. In patients treated with *in situ* breast cancer, the recurrence-rate of invasive tumor increased directly with ageing irrespective of tumor size or ER-status at diagnosis. Women who underwent lumpectomy without adjuvant radiation or chemotherapy exhibited significantly better overall and breast cancer specific survival rates than those receiving mastectomy, regardless of stage and ER-status of tumors. Antiestrogen treatment exhibited unforeseeable effectiveness even on targeted ER-positive tumors. Recent patents propose the detection of ESR1-gene amplification or restoration of ER-alpha expression for prediction of effective antiestrogen treatment, suggesting a crucial inhibitory role of estrogen-signaling against tumor-growth.

Conclusion: Estradiol-induced upregulation of estrogen signaling coupled with sparing of the estrogen-rich mammary fatpad are the most effective strategies against breast cancer.

Keywords: Antiestrogen resistance, breast cancer risk, DNA-stabilization, estradiol therapy, estrogen signaling, lumpectomy, mastectomy, protective adipocytes, radiation therapy.

INTRODUCTION

At the end of the 19th century, breast cancer regression was achieved by oophorectomy in premenopausal women with metastatic disease [1]. Similar observations led to the simplified erroneous concept that ovarian estrogen hormones may fuel the development of breast cancer, since estrogen deprivation by oophorectomy seemed to be an effective therapeutic means [2]. The tumor regression rate of 30% achieved by oophorectomy was not realized as being a very weak result, furthermore, it was highly questionable, which patient would exhibit good tumor response after the mutilating surgery.

Physicians overlooked the fact that living organisms are dynamic, self-regulating systems. Abrupt withdrawal of ovarian estrogen synthesis by means of oophorectomy provoked counteractive regulatory processes and intense hormone synthesis at many extragonadal sites of genetically proficient women [3]. Nevertheless, the low incidence rate of the anticancer effect of crude estrogen withdrawal indicated that the majority of breast cancer cases are not capable of extreme, compensatory reaction against the brutalism of oophorectomy. Estrogen deprivation and the achievement of "ideal" hypoestrogenic status in women as anticancer means gained great popularity among scientists and this enthusiasm led to an erroneous, unique pathway of breast cancer research throughout the 20th century until today.

In 1937, the next step on the route of endocrine manipulations was the development of diethylstilbestrol (DES), a synthetic estrogenic compound with anticancer capacity [4]; however, its therapeutic impact against breast cancer was

*Address correspondence to this author at the National Institute of Oncology, Surgical and Molecular Tumor Pathology Centre, Address: H-1122, Ráth György str. 7-9, Budapest, Hungary; Tel: 00 36 1 224 86 00; Fax: 00 36 1 224 86 20; E-mail: subazdr@gmail.com

2212-3970/16 $100.00+.00

similarly low as in case of oophorectomy. DES became a predominant agent in breast cancer care in spite of the inconsistent, weak therapeutic results and the experienced severe toxic side effects [4]. Considering its doubtful therapeutic value and high toxicity, DES was the first antiestrogenic compound, although it was designated as a direct stimulator of estrogen receptors (ERs).

In the early 70's, a synthetic ER blocker, tamoxifen was introduced for use in the treatment of ER-positive breast cancers [5] with the purpose to achieve the erroneous aim: appropriate defense against the presumably cancer promoter estrogen signaling. Preclinical and clinical data over decades have suggested that tamoxifen is not, as originally designated, a pure antiestrogen, but rather it may be regarded as a selective estrogen receptor modulator (SERM), having variable agonistic and/or antagonistic activities on ERs [6]. Tamoxifen treatment exhibited about 45-50% tumor response rate in breast cancer therapy even against the targeted ER-positive tumors [6]. The therapeutic dose of tamoxifen may cause severe, frequently life-threatening toxic effects, including thromboembolic complications, stroke [7] and malignancies at several sites [8], endometrial [9], gastric [10] and colorectal cancers [11] in particular. Moreover, *de novo* or acquired resistance against tamoxifen treatment induced refractoriness or advanced growth of tumors being mistakenly evaluated as an aberrant estrogenic activity of the compound [12].

In reality, tamoxifen is a pure ER blocker. Crude inhibition of the activation of ER-regulated genes provokes intense counteractions in genetically proficient patients, including extreme increase in new ER and estrogen synthesis. In these cases, the apoptotic activity of upregulated estrogen signaling results in transient tumor regression. Conversely, the long lasting fight between artificial ER blockade and a counteractive expression of ERs leads to the exhaustion of defensive mechanism. This Phase is clinically reflected by the stagnation or growth of breast cancer, coupled with toxic symptoms of estrogen deficiency. This Phase of complete ER-blockade is mistakenly regarded as antiestrogen resistance [3].

Later, further endocrine manipulations were carried out in order to successfully inhibit estrogen signaling by the administration of aromatase inhibitors designed to block the biosynthesis of estrogens [13]. Estrogen withdrawal provokes ER-overexpression even in tumor cells [14] and this acquired estrogen hypersensitivity restores estrogen signaling and may transiently promote tumor regression [3]. In the targeted postmenopausal patients, long-term aromatase inhibitor treatment leads to an exhaustion of extreme ER-protein synthesis resulting in tumor growth and toxic symptoms mimicking apparent aromatase inhibitor resistance. Since these compounds markedly suppress plasma estrogen levels via inhibition of the peripheral conversion of androgens to estrogens, the experienced aromatase inhibitor resistance may not be attributed to aberrant estrogenic activity [15].

Parallel with the erroneous struggle against estrogens as presumed carcinogenic agents for female breast, comprehensive research tried to reveal the secret of physiologic forces that safeguard the DNA-replication and ensure a long-lasting, tumor-free life in the vast majority of people and mammalians.

From the early 60's of the past century, the phenomenon of parity associated protection against breast cancer was observed among women from all ethnic groups. The risk of developing breast cancer was strongly reduced in parous women as compared with nulliparous cases [16-19]. Animal experiments in rats and mice also justified that pregnancies before or soon after exposure to chemical carcinogen are highly protective against the induction of mammary cancers [20, 21].

Administration of pregnancy mimicking high doses of estradiol and progesterone before or after carcinogen treatment provided strong protection against mammary carcinogenesis in rodents [22-26]. In ovariectomized female mice, heavy alcohol consumption and obesity increased the growth of experimental mammary tumors, while estrogen substitution promoted the loss of body fat, improved insulin sensitivity and suppressed the tumor growth [27-29]. The molecular bases of hormonal chemoprevention against mammary carcinogenesis were thoroughly considered as a new approach in the field of anticancer research [30, 31].

In the meantime, the erroneous fight against human breast cancer led to fairly doubtful preventive and curative results despite wide-spread mammographic screening and the continuous development of antiestrogenic agents by the pharmaceutical industry. Today, the possibilities of long term tumor recurrence and fatal outcome are not predictable even in case of breast cancers diagnosed and heavily treated at the earliest stage [32]. Moreover, women who had mastectomy or even surgical removal of both breasts, have higher risks of disseminated metastatic tumor spread as those having only a lumpectomy [33-35].

The unpredictable behavior of breast cancer strongly justifies that our standard approach to breast cancer therapy is erroneous. Breast cancer specialists should opt for something quite different than mutilating surgery, radiation, chemotherapy and antiestrogen administration.

IMPROVEMENT OF GENOMIC STABILITY MAY BE THE KEY TO HUMAN CANCER TREATMENT

DNA damages may originate from either endogenous or exogenous sources, while there is a wide range of physiologic mechanisms that help the surveillance and repair of DNA-replication [36]. In case of cancer diagnosis, the body's impaired defensive mechanisms should be recovered instead of attacking and killing the tumor cells by toxic chemicals or radiation.

Recently, novel therapeutic approaches have emerged against cancer initiation and spreading with the application of certain natural agents; such as phytochemicals, vitamin D and B, selenium, carotenoids, PARP inhibitors, and resveratrol [37, 38]. These new inexpensive, anticancer strategies with low toxicity may target the improvement of genomic stability.

FUNDAMENTAL ROLE OF ESTROGEN SIGNALING IN THE SOMATIC AND REPRODUCTIVE HEALTH OF MAMMALIANS

There are numerous mediators that create an enormous dynamic network for the supervision and repair of DNA-

replication. These interacting mediators, including transcription factors, receptors, enzymes and coregulators help to ensure the balance of all regulatory processes. In case of defective function or loss of any factor, all other players are recruited to induce compensatory reactions so as to restore the physiologic mechanisms. Estrogen bound ERs as transcription factors seem to be the crucial point of junction in this fairly complex regulatory network with innumerable possibilities for the occupancy of different promoter regions of abundant genes.

There are three types of estrogen hormones; estradiol (E_2), estrone (E_1) and estriol (E_3) of which the most effective and abundant is estradiol. Estrogen activated receptor isoforms; ER-alpha and ER-beta confer the estrogen signal by means of classic genomic and non-genomic pathways and ensure the surveillance of cellular health in both resting and proliferative biologic structures. ER-beta is mainly responsible for cell growth, while ER-alpha has crucial role in the regulation of cell proliferation and apoptotic cell death [39].

In the nucleus, ERs may act as ligand activated transcription factor proteins in the promoter region of target genes. They can also regulate gene expressions without binding to DNA via protein-protein interaction with nuclear transcription factors. Moreover, estrogen action also has rapid, non genomic signaling cascades via cell membrane associated ERs. The complexity of estrogen signaling includes not only liganded but also non-liganded activation of ERs [39].

Interplay between estrogen levels and ER expressions has crucial role in the maintenance of appropriate estrogen signaling, which is the prerequisite of somatic and reproductive health in mammalians. When estrogen signaling is jeopardized by genetic alterations and other endogenous or exogenous factors, defensive counteractions are recruited, such as increased ER expression and estrogen synthesis, while the improvement of estrogen signaling ensures safety DNA-replication [36, 40].

Both Low and High Estrogen Concentrations Upregulate ER-Expression and Transcriptional Activity

Both decreased and increased estrogen levels upregulate the ER expression and transcriptional activity of mammalian cells with the aim to maintain or enhance the crucial estrogen signaling [3].

Low estrogen level mean a high risk for breast cancer development [41, 42], while a counteractive overexpression of ERs may strengthen estrogen signaling and reduce the risk of malignancies [36]. In animal experiments, oophorectomy was found to abruptly decrease circulating estrogen concentrations; whilst highly increasing the levels of ER-alpha expression at different sites [43]. In women, benign, proliferative breast lesions frequently exhibit increased ER-expression [44], which may be a defensive counteraction against the dangers of low estrogen supply and cellular dedifferentiation [36].

In cases with serious mutations in human gene (CYP19) encoding aromatase P450, deficient conversion of androgens to estrogen leads to estrogen deficiency [45]. Androgen excess and estrogen loss induce progressive signs of virilization and polycystic ovaries in mutation carrier girls. Estrogen deficiency hampers all phases of glucose metabolism from insulin synthesis to intracellular glucose uptake leading to insulin resistance [46]. Moreover, a defective estrogen signaling inadequately interacts with the DNA-stabilizer systems and the defect of DNA-surveillance increases the risk of malignancies developing at different sites [36].

Extremely increased estrogen levels in pregnancy and the associated overexpression of ERs are physiologic examples of the crucial role of cellular estrogen surveillance [47]. In pregnancy, a self-generating upregulation of both estrogen signaling and DNA-stabilizer systems safeguards the DNA-replication of rapidly proliferating maternal and fetal structures [36]. Estrogen induced upregulation of ER-expression in pregnant women may explain why anticancer estrogen effects are prolonged and powerful in multiparous women.

Hyperestrogenism may occur as a compensatory process as well, when estrogen signaling is endangered by the decreased expression and/or transcriptional activity of ERs [40]. In breast cancer cases, accidentally occurring hyperestrogenism is mistakenly thought of as an etiologic factor of tumor development, while breast cancer risk is in direct correlation with defective estrogen signaling irrespective of serum estrogen levels.

High estrogen concentrations increase ER-expression in tumor cells as well. ER-positive breast cancer cell lines were treated by four types of estrogens and all four were reported to highly increase ER-expressions as compared with untreated controls [48]. The authors of the study erroneously regarded the estrogen induced overexpression of ERs as an increased proliferative domination instead of an apoptotic impact. By contrast, these observations support that the estradiol induced upregulation of ER expression may have a strengthening impact on apoptotic activity even in poorly differentiated, apparently ER-negative tumor cells.

Both Low and High ER Expressions Require Upregulated Estrogen Synthesis

Decreased ER-expression or the defective transcriptional activity of ERs is usually compensated by increased estrogen synthesis [40]. On the other hand, high ER-expression may be indicative of deficient estrogen signaling requiring compensatory increase in estrogen synthesis [36, 40].

Diverse naturally occurring mutagenic variants of both ER-alpha and ER-beta have been identified, however, only a few point mutations proved to be pathogenic in human tissue samples, including breast cancers [49]. In postmenopausal women, the dramatically decreasing serum estrogen levels may manifest the earlier hidden point mutations of ER-regulator genes and the defective estrogen signaling increase the risk of breast cancer development [40, 50].

Loss or mutation of BRCA1/2 genes results in a defect, inhibiting the expression and liganded transcriptional activity of ERs, while upregulating aromatase mediated estrogen synthesis [36]. BRCA mutation carriers frequently exhibit clinical symptoms of defective estrogen signaling, while the serum estrogen levels in such cases are compensatory increased [51, 52]. Stronger estrogen synthesis is usually effective in stimulating refractory ERs and may decrease breast cancer risk in cases with BRCA mutations [53].

Severe resistance of ER-alpha, attributed to the crude mutation of ESR1 gene, provokes sky-high compensatory estrogen levels, while clinical symptoms suggest estrogen deficiency [54, 55]. In a young male patient with serious ESR1 gene mutation, ER-alpha resistance was associated with insulin resistance, obesity and premature cardiovascular lesions despite the extremely high serum estrogen levels [54]. In an ESR1 gene mutation carrier young girl, delayed puberty was observed with the misleading symptoms of estrogen deficiency, whilst exhibiting extremely high compensatory estrogen levels [55].

Tamoxifen blockade of ERs induces artificial estrogen resistance provoking extreme estrogen synthesis. The estradiol concentrations in the breast of premenopausal women taking tamoxifen was 8.2 times higher that observed in the breast of healthy cycling women, while 17.3 times higher that observed in postmenopausal women taking tamoxifen [56]. These findings explain that the transient anticancer effect in tamoxifen-treated premenopausal patients may be attributed to counteractive increase in estradiol concentration.

In breast cancer cases, of all tumor markers, ER-negativity is the strongest predictor of poor prognosis and fatal outcome of the disease [57, 58]. In women, the stronger the defect of estrogen signaling, the higher is the risk of poorly differentiated, ER-negative breast cancer development, irrespective of serum estrogen concentrations [53].

In physiologic pregnancy, rapid fetal cell proliferation and differentiation multiply ER expression and promotes exponentially increasing estrogen concentrations. Estrogen binding transforms ERs to ligand activated transcription factor proteins, which are capable of occupying promoter regions on selected target genes. Continuously increasing estrogen levels and ER expressions are indispensable during the whole fetal development [36].

In women, anovulatory infertility and nulliparity are associated with increased risk for breast cancer [42, 53]. Increased ER expression in the healthy female breast is an indicator of challenged estrogen signaling and a need for higher estrogen concentrations. In the breast of nulliparous women, significantly higher ER-alpha expressions were detectable as compared with the resting breast of parous subjects [59].

Benign proliferative breast lesions in patients later experiencing breast cancer development exhibited significantly higher ER-alpha expression as compared with the lesions of controls who remained cancer-free [44]. It can be concluded that benign breast lesions with high ER-alpha expressions are indicators of defective estrogen-signaling and breast cancer risk.

THE IMPORTANCE OF LOCAL ESTROGEN SYNTHESIS IN THE BREAST

Mammary lobules and ducts are embedded in abundant adipose connective tissue, providing massive mechanical support and energy supply for the glandular structures. The adipocytes in the fatty tissue mass of the female breast also exhibit cyclic changes. The hormone sensitivity of adipocytes plays pivotal role in changes in energy homeostasis, lipid metabolism, insulin sensitivity and immune responses, while they are capable of the secretion of adipokines and further hormones, such as estrogens [60, 61].

Aromatase Enzyme Synthesis of Mammary Adipocytes Provides a Safeguard for the Cyclic Proliferative Activity of Glandular Breast Tissue

In premenopausal women, the ovaries are the main sources of estrogen synthesis. By contrast, in healthy postmenopausal women, serum estrogen levels are fairly decreased, whereas local estrogen concentrations in the breast are much higher, quite similarly to the premenopausal levels [61, 62].

The aromatase synthesizing capacity of mammary adipocytes plays a crucial role in the safeguarding of DNA-replication in glandular mammary structures. Rapid local responses to decreasing levels of circulating estrogens have a great role in the maintenance of estrogen concentration in healthy breast tissue [63]. Mammary adipocytes are recruited and activated by decrease in the circulating estrogen levels. The activated adipocytes are transformed from lipid laden cells into adipose fibroblasts, which are capable of increased aromatase synthesis [64-66]. In this way, the decrease in systemic estrogen supply may be locally counteracted by the increased conversion of androgens to estrogens.

In obese postmenopausal women, the maintenance of high estrogen concentrations in healthy breasts is mistakenly regarded as possibility for a high rate of cancer development in spite of the low circulating estrogen levels [63, 67].

Protective Estrogen Synthesis of Adipocytes in the Microenvironment of Breast Cancer

Local aromatase activities were measured in the different quadrants of tumor-bearing mastectomy specimens. The enzyme activity was always higher in the tumor containing quadrant, which in turn proved to be double as compared with the enzyme expression detectable in the cancer-free parts of the breast [61].

High aromatase activity and elevated local estrogen concentrations were particularly demonstrated at the invasive front of cancers where the tumor-stromal interactions define the fate of cancer cells [68, 69]. Studies on the molecular interactions between breast cancer cells and fibrous adipocytes revealed that malignant epithelial cells secrete significant quantities of prostaglandin E_2 (PGE_2), which stimulates extreme aromatase expression [70]. Both accumulation of aromatase synthesizing fibrous adipocytes and increased estrogen level close to invasive cancer cells are mistakenly regarded as essential inducers of breast cancer proliferation and invasion [64, 70, 71].

Recent literary data suggest that increased in-breast aromatase activity may have protective impact against breast cancer proliferation and spread. In young premenopausal cases with breast cancer, clinical control and the examination of removed tumor samples revealed that the absence of CYP19-aromatase activity carried a significantly high risk for local tumor recurrence and poor prognosis [72]. In se-

quential biopsies, the measurement of aromatase concentration showed markedly rising trend in enzyme activity during the effective aromatase inhibitor treatment of advanced primary breast cancers [73]. These findings strongly support that the aromatase inhibitor treatment may induce tumor regression by counteractive increased aromatase activity and estrogen synthesis [3].

In postmenopausal patients with advanced or recurrent ER-positive breast cancer, exhaustive aromatase inhibitor treatment induced antiestrogen resistance and tumor growth. High dose estrogen administration successfully suppressed the growth of tumors after prior exhaustive aromatase inhibitor treatment [74]. Recently, in clinical practice, physiologic estrogen induced apoptosis is successfully applied for breast cancer prevention and treatment following estrogen withdrawal [75].

In conclusion, increased aromatase activity of mammary adipocytes and local estrogen synthesis are recruited at the interface of tumor and fatty tissue for the inhibition of tumor proliferation.

MOLECULAR MECHANISMS OF THE TRANSIENT UPREGULATION OF ESTROGEN SIGNALING BY ANTIESTROGEN TREATMENT

Among other functions, estrogen signaling is the chief safeguard of genome stability in strong interplay with DNA-controlling and repairing systems, such as BRCA-genes and their protein products [36]. The crucial significance of estrogen signaling underlines the fact that any attack against the transcriptional activity of ERs results in an emergency situation, since it endangers the life of patients and requires intense counteractions.

Antiestrogen treatment, by either ER or aromatase inhibition entails a similar blockade of crucial estrogen signaling like severe genetic mutations. The counteractive upregulation of the circle of estrogen signaling and DNA-stabilizer systems depends on the genetic reserve capacities of the patient. In genetically proficient cases the antiestrogen attacks completely upregulate the whole system and a transient tumor regression may be experienced without toxic symptoms. These cases may comprise the magic 30% of breast cancer patients who react favorably to antiestrogen treatment. Nevertheless, this upregulative action is fairly vulnerable, since abundant protein synthesis associated with the overexpression of numerous biologic mediators may be exhausted during the continuous dosing of poisonous substance. This exhaustive phase is mistakenly regarded as acquired antiestrogen resistance [3].

The molecular mechanisms of the apparent anticancer effect of antiestrogens are based on the continuous extreme upregulation of both estrogen and ER synthesis (Fig. **1**). The aromatase inhibitor enters the circle of estrogen synthesis in a crude manner and there is a rapid drop in estrogen concentrations resulting in estrogen deficiency. The ER inhibitor binding on ERs radically decreases the amount of available ERs, which than manifests in estrogen resistance. The antiestrogen attack provokes prompt counteractions. Endangered estrogen signaling induces a rapid occupation of ESR1 promoter region by E2-activated ERs. Newly synthesized, abundant amounts of ER-alpha bind estrogen ligand and exert its transcriptional activity on the BRCA promoter region and increases BRCA1 protein synthesis. This important step enhances and restores the surveillance of safety DNA replication. An abundance of BRCA1 protein is capable of occupying the CYP19A promoter region and increasing the expression of aromatase A450 enzyme. Increased aromatase activity rapidly transforms androgens to estrogens and results in an elevated degree of estrogen concentration at several sites, in the tumor bearing breast in particular. Increased estrogen concentration activates the newly synthesized, plentiful ER-alpha, which occupies again the ESR1 promoter region and induces further increased expression of ER-alpha mRNA and new ER-alpha protein synthesis.

In genetically proficient women, this autoregulative circle leads to extreme upregulation of estrogen signaling and at the same time continuously improves the safeguarding of DNA replication. By this way the antiestrogen treatment may result in transient tumor regression [3].

Conversely, in the majority of patients with an insufficient upregulative capacity of estrogen signaling or those suffering from exhaustive antiestrogen treatment, a completion of the antiestrogen blockade of estrogen signaling leads to unrestrained tumor growth and metastatic spread [3]. Successful antiestrogen binding of ER-alpha in receptor positive tumors is a chemical blockade of possible transcriptional activities on estrogen regulated genes. In reality, antiestrogens create artificial ER-negative tumor cells from ER-positive ones. This Phase is mistakenly regarded as antiestrogen resistance.

High dose estrogen treatment is capable of restoring estrogen signaling despite heavy exposure to antiestrogen therapy by means of promoting an abundance of new ER expressions [3]. In animal experiment, a 9-week raloxifene or tamoxifen treatment strongly stimulated the growth of antiestrogen resistant MCF-7/Ral tumor, while it was highly ER-positive [76]. The tumor growth stimulation by antiestrogens was followed by a 5-week estradiol treatment, which statistically significantly reduced the size of tumors. The results of this experiment suggest that either antiestrogens or estrogens are capable of upregulating the ER expression and there is a competition for binding on the newly formed receptors. The predominance of antiestrogen binding on abundant ERs promotes the proliferative activity of tumors, whereas principally estradiol-bound ERs reactivate apoptotic pathways resulting in tumor regression [3].

ATTEMPTS TO PREDICTION AND IMPROVEMENT THE ANTIESTROGEN SENSITIVITY OF BREAST CANCERS

Recent publications have recommended new methods to predict the antiestrogen sensitivity of newly diagnosed tumors and to overturn the apparent antiestrogen resistance in cases of advanced cancer showing metastatic spread. Although, antiestrogen therapy targets the inhibition of ER

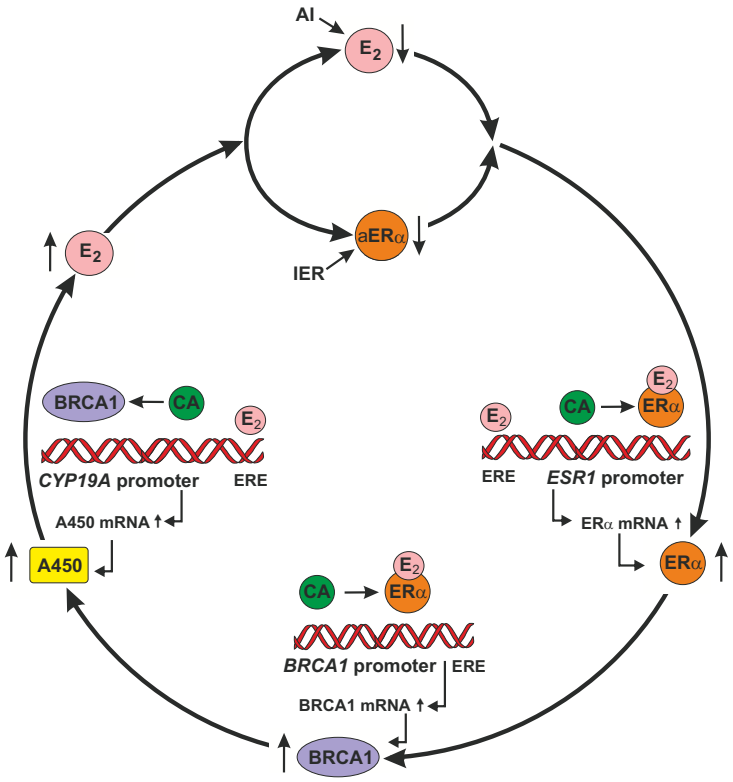


Fig. (1). Self-generating upregulation circle of estrogen signaling and DNA-safeguarding by antiestrogen treatment in genetically proficient women.

Aromatase inhibitor (AI) induces dramatic decrease in estradiol ($E_{2)}$ level, while the inhibitor of estrogen receptor (IER) blocks estrogen receptor alpha (ERα) strongly reducing the amount of accessible receptors (aERα). The emergency situation provokes rapid binding of E_2 by ERα and the complex occupies ESR1 promoter region resulting in upregulation the expressions of both ERα mRNA and ERα protein. In turn, elevated ERα level upregulates expressions of BRCA1 mRNA and BRCA1-protein, increasing the capacity for DNA stabilization. High BRCA1-protein levels further upregulate E_2 synthesis by means of occupation of CYP19A promoter and increasing expressions of both A450 mRNA and aromatase enzyme (A450). This cyclic mechanism ensures a continuous upregulation of estrogen signaling as a counteraction against antiestrogen treatment.

Abbreviations: CA: Coactivator; ERE: Estrogen Responsive Element

transcriptional activity, endocrine therapy resistance seems to be in close correlation with defective ER function and/or mutagenic ESR1-gene alterations. At the same time, upregulation and/or restoration of estrogen signaling seem to be advantageous for the effectiveness of antiestrogen treatment against tumors (Table **1**).

The increased estrogen receptor content of breast tumors was in significant correlation with prolonged survival both for premenopausal and postmenopausal women [77]. Adjuvant tamoxifen treatment could not further increase the survival of patients with strongly ER-positive tumors as compared with control patients with similar ER-expressions.

Decreased or altered ER-alpha phosphorylation was supposed to be associated with endocrine therapy resistance in breast cancer models, in spite of the overexpression of ER alpha [78, 79]. In total, 19 phosphorylation sites have been identified in ER alpha, defining different transcriptional activities. Preserved phosphorylation capacity at S167, S118, and S282 sites is beneficial for tamoxifen-induced tumor regression according to reported experimental and clinical data. By contrast, tamoxifen resistance associated tumor growth is likely to occur when S104/S106 or S305 is preferentially phosphorylated [79].

The grade of phosphorylation activity at Ser167 site of ER-alpha in breast tumors of tamoxifen-treated patients proved to be an excellent predictor of a good prognosis of the disease [80]. Since Ser167 phosphorylation is the most important element of the physiologic transcriptional activity of ERs, this result justified that compensatory maintenance of appropriate ER signaling defines longer disease-free and overall survival even among antiestrogen-treated breast cancer patients.

ESR1-gene mutations affecting the ligand-binding domain of ER alpha were presumed as crucial mechanisms in the acquired antiestrogen resistance of metastatic breast cancers [81]. Point mutations of ESR1 gene are relatively frequent and result in slightly variant forms of ERs. Gene polymorphism studies revealed that postmenopausal estrogen loss may amplify the earlier hidden slight genetic defects of ERs being associated with risk for diverse diseases, such as breast cancer [46, 82]. There may be a strong parallelism between postmenopausal estrogen loss and the antiestrogenic

Table 1. Reports Supporting the Upregulation of Estrogen Signaling in the Background of Apparently Successful Antiestrogen Treatment.

Antiestrogen Treatment	Aromatase Activity	Estradiol Concentration	ER-alpha Expression	ER-alpha Phosphorylation	ESR1-gene Amplification
Aromatase inhibitor (ref. 72)	↑				
Aromatase inhibitor (ref. 73)	↑				
Tamoxifen (ref. 56)		↑			
Tamoxifen (ref. 77)			↑		
Tamoxifen and AI. (ref. 90)			↑		
Tamoxifen (ref. 80)				↑	
Tamoxifen (ref. 83)					↑
Tamoxifen and AI. (ref. 89)					↑

inhibition of estrogen signaling, resulting in manifested defects of estrogen surveillance in ESR1-gene mutation carriers [3].

ESR1-gene amplification is associated with overexpression of ER-alpha. ESR1 amplification was frequently found in proliferative breast diseases and seems to be a characteristic finding in highly ER-positive breast cancers [83]. In breast cancer cases who had received adjuvant tamoxifen monotherapy, survival was significantly longer for women with cancers exhibiting ESR1 amplification than for women with cancers without ESR1 amplification.

Recent patents recommend various methods to predict the antiestrogen sensitivity of cancers and provide possible solutions to prevent or overturn antiestrogen resistance.

There is a recent patent providing a selective inhibitor of CDK8/19 for the treatment of patients with ER-positive breast cancer in combination with antiestrogen therapy, including tumors that are resistant to endocrine treatment [84]. CDK8 could act as a positive effector of ERs, thereby enabling tumor cells with low ER expression to utilize the estrogen signal more efficiently. The efficacy of using CDK8/19 inhibitor in breast cancer treatment is an important observation; while the real mechanism may be a counteractive upregulation of ER signaling instead of the additional blockade of ERs as suggested by the inventor.

A recent invention reveals that the overexpression of HOXB7 gene in ER-positive breast cancer cells confers tamoxifen resistance. Since homeobox genes are encoding nuclear proteins that act as transcription factors during normal cell development and differentiation, the inventor erroneously regards the elevation of HOXB7 expression as a key step in the acquisition and maintenance of tamoxifen resistance in breast cancers [85]. Nevertheless, the increased HOXB7 expression in tamoxifen resistant cancer cells seems to be an insufficient defensive counteraction rather than having a causal role.

A new patent provides treatment for MUC1+/ERα+/- cancers, particularly in case of tamoxifen resistance. Special MUC1 peptides have been shown to inhibit growth and promote death of MUC1-expressing tumor cells. These peptides can be used advantageously in combination with ER-targeted therapy even against ER-overexpressing, antiestrogen-resistant tumors [86].

A new innovation provides an agent capable of reverting tamoxifen-resistant tumor cells to a tamoxifen-sensitive phenotype for the treatment of breast cancer. This compound decreases cellular expression of at least one of miRNAs that are up-regulated in tamoxifen-resistant (TR) cells or increases cellular expression of at least one of miRNAs that are down-regulated in TR cells [87].

A recent disclosure provides methods for assaying the mutations of ESR1 gene at different domains and for the analysis of mutation associated ER protein variants influencing the effectiveness of treatment course. Breast cancers with acquired ESR1 gene mutations maintained sensitivity to antiestrogen therapy, particularly in tumors developing resistance to aromatase inhibitors associated with ESR1 amplification reactions [88]. This finding justifies that acquired ESR1 gene mutations may further enhance ER expression and maintain its apoptotic activity when ESR1 amplification is no longer enough for the restoration of ER signaling.

Certain patents reasonably suggest that antiestrogen effectiveness may be associated with the capacity for upregulation of estrogen signaling. A proposed *in vitro* method for detection of ESR1 gene amplification in breast cancers is capable of identifying candidate patients with a proliferative benign or malignant breast disease as suitable for antiestrogen treatment [89]. Use of a Wnt-5a protein or a peptide thereof, possessing Wnt-5a signaling properties, enables restoration of ERα expression and makes it possible to treat resistant breast cancers with selective estrogen receptor modulators, such as tamoxifen, or aromatase inhibitors (Table **1**) [72-90].

UNPREDICTABLE RECURRENCE OF BREAST CANCERS EVEN DETECTED IN THE EARLY STAGES REQUIRES CHANGES IN STANDARD THERAPEUTIC MEASURES

The results of current therapeutic procedures against mammary malignancies are strongly unpredictable despite the continuous improvements in methods for detection and treatment measures in the developed countries [91]. A puzzling experience in relation to breast cancer care is that approximately one-third of women initially diagnosed with early stage breast cancer eventually develop recurrent advanced disease with metastases and fatal outcome, no matter which treatment procedure they had [92].

A unique, vicious aspect of current breast cancer treatment is that it does not achieve the usual five-year tumor-free survival as a milestone of definite healing as is regularly experienced in case of many other malignancies. Nowadays, breast cancer cases may not be considered as being cured until the patients die of some other causes.

Breast cancer cases treated with mastectomy were more likely to die from cardiovascular complications, venous thromboembolism and other diseases in the first three years following surgery when compared with women who had lumpectomy [33, 93]. In breast cancer patients, tamoxifen treatment was associated with fourfold higher risk of venous thromboembolism as compared with risk before starting therapy [94]. In patients with early breast cancer, adjuvant radiotherapy increased the risk of ischemic heart disease [95], moreover, an increased long term risk of second primary solid cancers was observed at radiotherapy associated sites [96].

These experiences strongly suggest that we have not been treating breast cancer cases in the right way, and quite new approaches to the prevention and treatment of these tumors are necessary.

Failures of the Treatment and Care of Cases with Ductal Carcinoma *In Situ*

The widespread mammographic screening is successful at identifying the earliest stage of breast cancer, the so-called 0 stage tumor, or ductal carcinoma in situ (DCIS) and nowadays DCIS accounts for approximately 20-25% of screen-detected breast cancers [97].

Symptom-free DCIS may be regarded as a transient equilibrium between proliferating tumor cells and local tumor cell killer activities. DCIS being left alone may remain occult for a long time, sometimes may exhibit spontaneous healing or may be transformed into invasive cancer depending on the activities of systemic and local defensive anticancer mechanisms.

Long term epidemiologic studies have demonstrated that the annual surgical treatment of 50 000 to 60 000 DCIS lesions has not reduced the overall number of invasive breast cancer cases and disease specific mortality rate [98]. Since early diagnosis in itself seems to be ineffective in the reduction of invasive breast cancer rate, quite new therapeutic measures and testing new approaches to care patients are necessary to achieve a decrease in breast cancer mortality [97, 99].

In a large prospective study including more than 100 000 women with DCIS diagnosis, it was established that ductal carcinoma in situ carries a higher risk of breast cancer-related death than previously thought [32]. Women diagnosed with DCIS are twice as likely to die compared to the general US population. The consistent incidence rate of invasive breast cancer deriving from DCIS justifies that the standard surgical and adjuvant therapy of high grade DCIS is not equivocally capable of ensuring a tumor-free life, while low grade DCIS is perhaps superfluously over treated [97, 99].

Among women who were diagnosed with DCIS, the risk of dying of breast cancer was 3.3% during the 20-year study period, while 5% of patients died of other causes [32]. These results suggest that the vast majority of women with very early stage breast cancers may not need aggressive surgical and radiation therapy.

Nevertheless, breast cancer specialists should not be assured by the low breast cancer-specific mortality rate associated with an apparently cured DCIS. Appearance of DCIS should be regarded as an early, localized indicator of a disturbed hormonal, metabolic and DNA-stabilizing equilibrium [99]. DCIS may be the predecessor not only of possibly invasive breast cancer but also of stroke, thromboembolic events, cardiovascular complications, type-2 diabetes and malignancies at other sites associated with the disturbed estrogen signaling. Appearance of other fatal diseases may easily precede the recurrent mammary malignancy, and the patient will die of other causes.

Pathologic Characteristics of DCIS Are Predictive For the Type of Developing Recurrent Cancer and May Define the Therapeutic Approach

The morphologic characteristics of DCIS often predict the type of cancer that may develop in the future; consequently, the exact pathologic diagnosis of DCIS should be

regarded as a clue to how we can specifically prevent the recurrence of an invasive, potentially lethal breast cancer [97]. High-risk DCIS type lesions are usually exhibiting ER-receptor negativity, strong HER2 positivity or a large size.

A clinical trial included 665 patients with DCIS treated by lumpectomy without radiation to establish the 12-year risk of breast cancer recurrence on the treated side [100]. Breast cancer recurrences were registered separately in low- or intermediate-grade DCIS cases (group 1); and in high-grade DCIS cases (group 2). The 12-year rates of developing a recurrent breast tumor were significantly lower, 14.4% for group 1 and surprisingly higher, and 24.6% for group 2. The rates of developing an invasive breast malignancy were 7.5% and 13.4%, respectively. The risk of developing DCIS and invasive cancer in the ipsilateral breast exhibited a continuous increase throughout the 12 years of follow-up, without any plateau.

These results strongly suggest that ageing related increase in the defect of estrogen signaling may be directly associated with recurrent breast cancer development, in spite of the surgical removal of tumors. Moreover, high grade DCIS proved to be an indicator of stronger systemic defect in estrogen signaling and significantly higher danger for the recurrence of both overall and invasive breast cancers as compared with low grade DCIS.

Increased Risk Of Breast Cancer Specific Mortality among Very Young and Dark Skinned American Women with DCIS

In cases, when DCIS is diagnosed before the age of 35-40 years, the young age seems to pose an increased risk of breast cancer recurrence [32]. Breast cancer specific mortality was higher for women who received a DCIS diagnosis before age 35 years compared with older women (7.8% vs 3.2%). Among women under age 35, the mortality rate was 17 times higher than average within 9 years of DCIS diagnosis. High risk characteristics of DCIS, such as hormone receptor negativity, Her2-positivity, and high-grade often overlap the lesions diagnosed at a young age [97].

Nevertheless, the real explanation for apparent danger of young age may be that young women with an active menstrual cycle enjoy the anticancer effect of even a mildly decreased circulating estrogen supply. The relatively high percentage of poorly differentiated breast cancer initiation among hormonally active young cases may be attributed to the low incidence rate of more successfully repressed ER-positive cancers rather than an excessive inclination to ER-negative tumors [53]. In young patients, the incidence of high risk, ER-negative DCIS indicates the strong disturbances of the hormonal balance requiring increased exogenous estrogen substitution so as to strengthen the DNA-stabilizer mechanisms [36, 40].

Among cases with DCIS, the African-American race of women is strongly associated with an increased rate of high risk characteristics of lesions [32, 97]. At 20 years, the breast cancer-specific mortality for blacks was 7.0% being much higher compared with 3.0% for non-Hispanic whites (HR, 2.55). Black women with a DCIS diagnosis face a lower survival rate (93%) over 20 years than other ethnic groups (97%) [32].

In dark-skinned American women, the higher risk of developing poorly differentiated breast cancers as compared with white patients may be explained by the incongruence between their excessive pigmentation and the poor light and sunshine exposure of North-America [53,101]. Natural light deficiency is associated with melatonin excess and further deleterious metabolic and hormonal alterations; such as deficiencies of estrogen, thyroxin and vitamin-D and deepening insulin resistance. All these disorders confer excessive breast cancer risk. Recognition of these correlations suggests that an intense care of the hormonal and metabolic equilibrium of African-American women may prevent and cure even high risk tumors and may effectively reduce their overall and breast-cancer specific mortality [101].

The Questions of the Indication of Radical Surgery and Radiotherapy for the Treatment of Breast Cancer

Today, breast cancer specialists are unable to predict either the probability of tumor recurrence or the life expectancy of patients, since the standard therapeutic approaches to breast cancer are inappropriate. Faced with a diagnosis of breast cancer, more and more women are choosing ipsilateral mastectomy or the removal of both breasts [102]. Nevertheless there are evidences supporting the highest survival rate of patients who underwent breast conservation surgery.

Survival rates after lumpectomy and mastectomy were compared in a large population-based study of 112,154 women diagnosed with stage I or stage II breast cancers, followed on average for 9.2 years [33]. Benefit of breast cancer-specific survival was greater after lumpectomy as compared with mastectomy among women over 50 years of age with ER-positive tumors (HR: 0.86), whereas this trend was quite similar in younger women, aged less than 50 years with ER-negative disease (HR: 0.88). Women who underwent lumpectomy exhibited better overall and breast cancer specific survival rates, regardless of patients' age or ER-status of the tumor.

Improved survival rate among patients receiving lumpectomy may be attributed to the preservation of mammary fat-pad containing estrogen synthesizing adipocytes and other defensive cellular factors.

The hazard of breast cancer-related death was analyzed after treatment by lumpectomy, mastectomy alone or mastectomy with radiation for women with early stage invasive ductal carcinoma in the period from 1998 to 2008 [34]. Of the 132149 patients included in the study, 70% were treated with lumpectomy, 27% with mastectomy and 3% with mastectomy and radiation. The 5-year breast cancer-specific survival rates of patients who underwent lumpectomy, mastectomy alone, or mastectomy with radiation were 97%, 94% and 90%, respectively. The 10-year breast cancer-specific survival rates were much lower, particularly in the third group; 94%, 90%, and 83%, respectively. These results reveal that among cases with early stage ductal carcinoma, patients who underwent lumpectomy had a higher breast cancer-specific survival rate than those treated with either

mastectomy alone (HR: 1.31) or mastectomy with radiation (HR: 1.47).

The best life expectancy of patients receiving breast conserving surgery alone may be attributed to the intact fatty tissue pad of the preserved breast tissue. Mastectomy with radiation resulted in the lowest breast cancer specific survival rate, since mammary ablation extirpated the vast majority of local defensive cellular factors and radiation destroyed the remaining defensive cells, which were accidentally left behind.

In the study by Narod *et al.,* the ipsilateral and contralateral invasive breast cancer recurrences in cases with DCIS were similar; 5.9% and 6.2% respectively, while the contralateral recurrence of tumors was somewhat higher [32]. Contralateral recurrence of DCIS seems to be an indicator of a systemic risk for tumor initiation, particularly in the breasts, since cancer cannot jump from one breast to the other. The higher risk of tumor recurrence in contralateral breast may be explained by the lower concentration of defensive adipocytes as compared with the earlier tumor bearing ipsilateral breast.

Mortality rate following bilateral mastectomy was compared with other surgical interventions against breast cancer in California [35]. The participants were women diagnosed with stages 0-III unilateral breast cancer and the median follow-up was 7.7 years. Among 189 734 patients, the rate of bilateral mastectomy increased from 2.8% in 1998 to 12.3% in 2011. Rate of bilateral mastectomy among women younger than 40 years achieved 33% in 2011. The lowest ten-year mortality rate was 16.8% among cases receiving breast conserving surgery with radiation, while unilateral and bilateral mastectomy were associated with higher mortality rates; 18.9% and 20.1%, respectively. These findings exhibit that bilateral mastectomy resulted in the highest rate of mortality among patients treated by surgery.

After a decades-long trend toward the practice of less invasive surgery, it can be established that there is no sense in performing mastectomy, contralateral preventive mastectomy or radiotherapy even in cases with high risk breast cancers, since extended mutilating surgery and radiation rather deteriorates the survival expectancy of patients.

Development of Disseminated Metastases without a Detectable New Primary or Recurrent Breast Cancer

Many years or decades after the diagnosis and treatment of primary breast tumor, in about 20-40% of patients, disseminated metastases develop without new primary or recurrent cancer lump detection in the breast [103]. Dormancy of tumor cells was supposed as a hypothetic mechanism leading to occult micrometastases attributed to the spread of residual tumor cells that were left after an apparently successful removal of the primary tumor. Nevertheless, mechanisms that regulate the transition of disseminated tumor cells from dormant into a proliferative state remain unknown [104].

Among 517 women initially diagnosed with DCIS and dying of metastatic breast cancer over a period of 20 years, less than half (41.3%) experienced invasive recurrence of a breast tumor prior to death, while the majority (54.1%) died without a detectable invasive in-breast tumor recurrence [32]. Among DCIS cases, which were treated with lumpectomy and radiotherapy and then died of breast cancer, 57.7% did not experience an in-breast cancer, while among women receiving lumpectomy alone 50.0% did not exhibit an in-breast tumor prior to death [32].

In conclusion, women treated with adjuvant radiotherapy, had higher risk for unrestrained tumor spread without a local invasive recurrence of breast cancer. These experiences aroused an important question: How could the women have died of disseminated metastatic breast cancer without having a detectable, local invasive tumor recurrence?

In certain patients with apparently cured DCIS lesions, the deepening systemic hormonal and metabolic imbalance may be coupled with the exhaustion of hormonal and immunologic defense reactions within the breast. The more unarmed the microenvironment of mammary cells, the higher is the possibility of initiation and unrestrained, disseminated proliferation of breast cancer cells without the development of a somewhat suppressed early-stage invasive in-breast cancer.

Consistent findings have shown that women who have undergone mastectomy have a higher risk of breast cancer related death as those who had only a lumpectomy [33-35]. Even after careful mastectomy, a few residual ductal and/or lobular cells may be left behind. Age related continuous decrease in circulating estrogen levels may induce new cancer initiation from the persistent epithelial cells remaining after mammary ablation. Moreover, the loss of estrogen synthesizing defensive fatpad provides desolated tissue environment, which is favorable for the unrestrained proliferation and rapid disseminated spread of cancer cells. In conclusion, the radical mutilating surgery in breast cancer cases is not safety but rather provides high risk for aggressive metastatic tumor propagation without formation of a detectable local tumor lump.

CONCLUSION

Development of cancer in the breast is a local manifestation of the systemic defect of estrogen surveillance associated with imbalances of DNA-stabilizing processes. The female breast is extremely vulnerable to the defective safeguarding of estrogen signaling attributed to its cyclic proliferative activity. The grade of defect in estrogen surveillance is directly associated with the aggressive biologic behavior and poor differentiation of developing breast malignancies in both young and older patients.

Age related decrease in estrogen supply, particularly after menopause, may be directly associated with increased inclination to recurrent breast cancer development, in spite of extensive surgical and standard adjuvant therapy of tumors.

Surgical removal of breast tumors by lumpectomy shows the best long term therapeutic results by reason of preservation of the estrogen rich fatpad located in the neighborhood of the tumor. Adjuvant radiotherapy or chemotherapy may worsen the possibility of tumor-free survival by weakening the local and systemic defense reactions of patients. By contrast, natural estrogen therapy is capable of strengthening all defensive mechanisms, while killing residual or accidentally initiated tumor cells.

Adjuvant antiestrogen therapy used for prevention of tumor recurrence is a double-edged sword. The extreme upregulation of estrogen signaling by antiestrogens may easily become exhausted even in genetically proficient women. Moreover, in genetically challenged cases, antiestrogens induce rapid blockade of estrogen regulated genes and promote tumor recurrence in addition to toxic symptoms.

Breast cancer is curable. Breast conservation surgery should be coupled with systemic improvement of estrogen signaling and the associated DNA-stabilizer processes by means of natural estrogen treatment aiming the prevention of either recurrent or newly initiated breast tumor.

CURRENT & FUTURE DEVELOPMENTS

Today, the behavior of breast tumors diagnosed even in the earliest stage remains unpredictable regardless the use of any standard therapeutic measures. These failures strongly suggest that either the classic aggressive methods of cancer therapy or the unique pathway of antiestrogen attack used against breast cancer may be erroneous.

Improvement of estrogen signaling by exogenous estrogen administration is the causal therapy against breast cancer. Natural estrogen preparations are quite safety, however, the choice of safe estrogenic compounds from pharmaceutical products is not easy. Since antiestrogenic drugs may also have deceiving estrogen-like effects, there is great confusion concerning the recognition of the real properties of synthetic estrogens. The example of DES demonstrates that a highly toxic compound with weak anticancer capacity was used as an estrogen, while the clinical experiences on DES therapy clearly justified its antiestrogenic nature. Today, synthetic versions of 17-beta-estradiol are available; however it is yet to be clarified whether or not the biologic effects of these compounds are convincingly equal to those of physiologic estradiol or rather they are manipulated with the aim to reduce the mistakenly presumed carcinogenic effect.

The dose of effective estrogen therapy depends on the grade of decreased estrogen signaling. Complex evaluation of the deficiency of in-breast estrogen supply and the defective expression and transcriptional activity of ERs in the mammary cells may basically define the necessary estrogen administration, irrespective of the serum estrogen levels. In cases showing serious ER resistance, the necessary therapeutic estrogen dose may be high even though the patients may have hyperestrogenism, which is insufficient to restrain the growth of tumor.

The ER-expression level of breast tumors may also be an indicator of estrogen demand. ER-negative breast cancers are not distinct entities requiring quite different therapeutic measures, given that high dose estrogen is capable of inducing tumor differentiation, ER-expression and apoptotic death of tumor cells. The lower the ER expression of a breast cancer the higher is the effective therapeutic dose of natural estrogen without the risk of any toxic side effects.

Recent patents have developed methods to determine endocrine-resistance in tumor cells of patients who previously received antiestrogen therapy, or to determine the risk of patients who have not yet received tamoxifen therapy to become tamoxifen-resistant. New inventions also provide compounds, which are capable of converting tamoxifen resistant tumor cells to tamoxifen-sensitive phenotypes. These methods in turn may even be quite useful in planning individual estrogen therapy for each breast cancer patient.

Certain patents suggest new reasonable methods for the estimation of ESR1 gene aptitude and ER expression and possibilities for the upregulation of estrogen signaling. These manipulations may be essential for the improvement of therapeutic possibilities even in genetically challenged patients.

Breast malignancies are not capable of late recurrences after 15-20 years. These tumors may be regarded as newly appearing ones in either the ipsilateral or contralateral breast, attributable to the age-related, deepening defects of estrogen supply and the weakening defensive counteractions. Breast cancer cases should not be left alone after apparently successful therapy; they require strict control and maintenance of estrogen signaling over an entire lifetime.

Disclosure of the details of estrogen based therapy for cancer prevention and cure will require long lasting hard work; however, we should set about applying it in practice as soon as possible.

CONFLICT OF INTEREST

The author confirms that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

The author thanks her colleague; Dr. Károly Sándor Tóth gynecologist, the scientific leader of Hungarian Menopause Society, for sharing his clinical experiences and doubts regarding current endocrine therapy measures.

REFERENCES

[1] Beatson GT. On the treatment of inoperable cases of carcinoma of the mamma: Suggestions for a new method of treatment, with illustrative cases. Lancet 1896; 2: 104-7.

[2] Boyd S. On oophorectomy in cancer of the breast. BMJ 1900; 2: 1161-87.

[3] Suba Z. The pitfall of the transient, inconsistent anticancer capacity of antiestrogens and the mechanism of apparent antiestrogen resistance. Drug Des Devel Ther 2015; 9: 4341-53.

[4] Haddow A, Watkinson JM, Paterson E, Koller PC. Influence of synthetic oestrogens on advanced malignant disease. BMJ 1944; 2(4368): 393-8.

[5] Jordan VC, Dowse LJ. Tamoxifen as an antitumour agent: Effect on oestrogen binding. J Endocrinol 1976; 68: 297-303.

[6] Hayes DF. Tamoxifen: Dr. Jekyll and Mr. Hyde? J Natl Cancer Inst 2004; 96: 895-7.

[7] Braithwaite RS, Chlebowski RT, Lau J, George S, Hess R, Col FN. Meta-analysis of vascular and neoplastic events associated with tamoxifen. J General Int Med 2003; 18: 937-47.

[8] Schaapveld M, Visser O, Louwman MJ, de Vries EGE, Willemse PHB, Otter R, *et al*. Risk of new primary nonbreast cancers after breast cancer treatment: A Dutch Population-Based Study. J Clin Oncol 2008; 26(8): 1239-46.

[9] Chen JY, Kuo SJ, Liaw YP, Avital I, Stojadinovic A, Man YG, *et al*. Endometrial cancer incidence in breast cancer patients correlating with age and duration of tamoxifen use: A population based study. J Cancer 2014; 5(2): 151-5.

[10] Camargo MC, Goto Y, Zabaleta J, Morgan DR, Correa P, Rabkin CS. Sex hormones, hormonal interventions, and gastric cancer risk: A meta-analysis. Cancer Epidemiol Biomarkers Prev 2012; 21(1): 20-38.

[11] Newcomb PA, Solomon C, White E. Tamoxifen and risk of large bowel cancer in women with breast cancer. Breast Cancer Res Treat 1999; 53(3): 271-7.
[12] Larionov AA, Miller WR. Challenges in defining predictive markers for response to endocrine therapy in breast cancer. Future Oncology 2009; 5(9): 1415–28.
[13] Dixon JM. Endocrine resistance in breast cancer. New J Science 2014; ID 390618, 27 pp.
[14] Santen RJ, Martel J, Hoagland M, Naftolin F, Roa L, Harada N *et al.* Stromal spindle cells contain aromatase in human breast-tumors. J Clin Endocrin Metab 1994; 79: 627-632.
[15] Lin NU, Winer EP. Advances in adjuvant endocrine therapy for postmenopausal women. J Clin Oncol 2008; 26(5): 798-805.
[16] MacMahon B, Cole P, LIn TM, Lowe CR, Mirra AP, Ravnihar B *et al.* Age at first birth and breast cancer risk. Bull WHO 1970; 43: 209-21.
[17] Henderson BE, Powell D, Rosario I, Keys C, Hanisch R, Young M, *et al.* An epidemiologic study of breast cancer. J Natl Cancer Inst 1974; 53(3): 609-14.
[18] Kelsey JL, Gammon MD, John EM. Reproductive factors and breast cancer. Epidemiol Rev 1993; 15: 36-47.
[19] Britt K, Anshworth A, Smalley M. Pregnancy and the risk of breast cancer. Endocrine-Related Cancer 2007; 14(4): 907-33.
[20] Sinha DK, Pazik JE, Dao TL. Prevention of mammary carcinogenesis in rats by pregnancy: Effect of full-term and interrupted pregnancy. Br J Cancer 1988; 57: 390-4.
[21] Medina D, Smith GH. Chemical carcinogen-induced tumorigenesis in parous, involuted mouse mammary glands. J Natl Cancer Inst 1999; 91: 967-9.
[22] Huggins C, Grand LC, Brillantes FP. Mammary cancer induced by a single feeding of polynuclear hydrocarbons, and its suppression. Nature 1961; 189: 204-7.
[23] Grubbs CJ, Peckham JC, McDonough KD. Effect of ovarian hormones on the induction of 1-methyl-1-nitrosourea-induced mammary cancer. Carcinogenesis 1983; 4: 495-7.
[24] Sivaraman L, Stephens LC, Markaverich BM, Clark JA, Krnacik S, Conneely OM, *et al.* Hormone-induced refractoriness to mammary carcinogenesis in Wistar-Furth rats. Carcinogenesis 1998; 19(9): 1573-81.
[25] Rajkumar L, Guzman RC, Yang J, Thordarson G, Talamantes F, Nandi S. Prevention of mammary carcinogenesis by short-term estrogen and progestin treatments. Breast Cancer Res 2004; 6(1): R31-7.
[26] McCormick GM, Moon RC. Effect of increasing doses of estrogen and progesterone on mammary carcinogenesis in the rat. Eur J Cancer 1973; 9(7): 483-6.
[27] Hong J, Holcomb VB, Kushiro K, Núñez NP. Estrogen inhibits the effects of obesity and alcohol on mammary tumors and fatty liver. Int J Oncol 2011; 39(6): 1443-53.
[28] Holcomb VB, Hong J, Núñez NP. Exogenous estrogen protects mice from the consequences of obesity and alcohol. Menopause 2012; 19(6): 680-90.
[29] Nkhata KJ, Ray A, Dogan S, Grande JP, Cleary MP. Mammary tumor development from T47-D human breast cancer cells in obese ovariectomized mice with and without estradiol supplements. Breast Cancer Res Treat 2009; 114(1): 71-83.
[30] Russo IH, Russo J. Mammary gland neoplasia in long-term rodent studies. Environ Health Perspect 1996; 104(9): 938-67.
[31] Jerry DJ. Roles for estrogen and progesterone in breast cancer prevention. Breast Cancer Res 2007; 9(2): 102.
[32] Narod SA, Iqbal J, Giannakeas V, Sopik V, Sun P. Breast cancer mortality after a diagnosis of ductal carcinoma in situ. JAMA Oncol 2015; 1(7): 888-96.
[33] Hwang ES, Lichtensztajn DY, Gomez SL, Fowble B, Clarke CA. Survival after lumpectomy and mastectomy for early stage invasive breast cancer: The effect of age and hormone receptor status. Cancer 2013; 119(7): 1402-11.
[34] Agarwal S, Pappas L, Neumayer L, Kokeny K, Agarwal J. Effect of breast conservation therapy vs. mastectomy on disease-specific survival for early-stage breast cancer. JAMA Surg 2014; 149(3): 267-74.
[35] Kurian AW, Lichtensztajn DY, Keegan TH, Nelson DO, Clarke CA, Gomez SL. Use of and mortality after bilateral mastectomy compared with other surgical treatments for breast cancer in California, 1998-2011. JAMA 2014; 312(9): 902-14.
[36] Suba Z. DNA stabilization by the upregulation of estrogen signaling in BRCA gene mutation carriers. Drug Design Devel Ther 2015; 9: 2663-75.
[37] Ferguson LR, Chen H, Collins AR, Connell M, Damia G, Dasgupta S, Malhotra M, *et al.* Genomic instability in human cancer: Molecular insights and opportunities for therapeutic attack and prevention through diet and nutrition. Semin Cancer Biol 2015; 35 Suppl: S5-S24.
[38] Jiang WG, Sanders AJ, Katoh M, Ungefroren H, Gieseler F, Prince M, *et al.* Tissue invasion and metastasis: Molecular, biological and clinical perspectives. Semin Cancer Biol 2015; 35 Suppl: S244-75.
[39] Maggi A. Liganded and unliganded activation of estrogen receptor and hormone replacement therapies. Biochim Biophys Acta 2011; 1812(8): 1054-60.
[40] Suba Z. Diverse pathomechanisms leading to the breakdown of cellular estrogen surveillance and breast cancer development: New therapeutic strategies. Drug Design Devel Ther 2014; 8: 1381-90.
[41] Suba Z. Interplay between insulin resistance and estrogen deficiency as co-activators in carcinogenesis. Pathol Oncol Res 2012; 18(2): 123-33.
[42] Suba Z. Circulatory estrogen level protects against breast cancer in obese women. Recent Pat Anticancer Drug Discov 2013; 8(2): 154-67.
[43] Mohamed MK, Abdel-Rahman AA. Effect of long-term ovariectomy and estrogen replacement on the expression of estrogen receptor gene in female rats. Eur J Endocrinol 2000; 142(3): 307-14.
[44] Shaaban AM, Sloane JP, West CR, Foster CS. Breast cancer risk in usual ductal hyperplasia is defined by estrogen receptor-alpha and Ki-67 expression. Am J Pathol 2002; 160(2): 597-604.
[45] Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K. Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. J Clin Endocrinol Metab 1995; 80(12): 3689-98.
[46] Suba Z. Low estrogen exposure and/or defective estrogen signaling induces disturbances in glucose uptake and energy expenditure. J Diabet Metab 2013; 4: 272-81.
[47] Schumacher A, Costa SD, Zenclussen AC. Endocrine factors modulating immune responses in pregnancy. Front Immunol 2014; 5: 196.
[48] Liu S, Ruan X, Schultz S, Neubauer H, Fehm T, Seeger H, *et al.* Oestetrol stimulates proliferation and oestrogen receptor expression in breast cancer cell lines: Comparison of four oestrogens. Eur J Contracept Reprod Health Care 2015; 20(1): 29-35.
[49] Herynk MH, Fuqua SA. Estrogen receptor mutations in human disease. Endocr Rev 2004; 25(6): 869-98.
[50] Zheng SL, Zheng W, Chang BL, Shu XO, Cai Q, Yu H, *et al.* Joint effect of estrogen receptor beta sequence variants and endogenous estrogen exposure on breast cancer risk in Chinese women. Cancer Res 2003; 63(22): 7624-9.
[51] Widschwendter M, Rosenthal AN, Philpott S, Rizzuto I, Fraser L, Hayward J, *et al.* The sex hormone system in carriers of BRCA1/2 mutations: A case-control study. Lancet Oncol 2013; 14(12): 1226-32.
[52] Kim J, Oktay K. Baseline E (2) levels are higher in BRCA2 mutation carriers: A potential target for prevention? Cancer Causes Control 2013; 24(3): 421-6.
[53] Suba Z. Triple-negative breast cancer risk in women is defined by the defect of estrogen signaling: Preventive and therapeutic implications. Onco Targets Ther 2014; 7: 147-64.
[54] Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, *et al.* Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med 1994; 331: 1056-61.
[55] Quaynor SD, Stradtman EW, Kim HG, Shen Y, Chorich LP, Schreihofer DA, *et al.* Delayed puberty and estrogen resistance in a woman with estrogen receptor α variant. N Engl J Med 2013; 369(2): 164-71.
[56] Depypere HT, Bolca S, Bracke M, Delanghe J, Comhaire F, Blodeel P. The serum estradiol concentration is the main determinant of the estradiol concentration in normal breast tissue. Maturitas 2015; 81(1): 42-5.
[57] Hartley MC, McKinley BP, Rogers EA, Kalbaugh CA, Messich HS, Blackhurst DW, *et al.* Differential expression of prognostic factors and effect on survival in young (< or =40) breast cancer patients: A case-control study. Am Surg 2006; 72(12): 1189-94.
[58] Talley LI, Grizzle WE, Waterbor JW, Brown D, Weiss H, Frost AR. Hormone receptors and proliferation in breast carcinomas of

equivalent histologic grades in pre- and postmenopausal women. Int J Cancer 2002; 98(1): 118-27.
[59] Asztalos S, Gann PH, Hayes MK, Nonn L, Beam CA, Dai Y*, et al*. Gene expression patterns in the human breast after pregnancy. Cancer Prev Res (Phila) 2010; 3(3): 301-11.
[60] Pallottini V, Bulzomi P, Galluzzo P, Martini C, Marino M. Estrogen regulation of adipose tissue functions: Involvement of estrogen receptor isoforms. Infect Disord Drug Targets 2008; 8(1): 52-60.
[61] Simpson ER. Sources of estrogen and their importance. J Steroid Biochem Mol Biol 2003; 86(3-5): 225-30.
[62] Van Landeghem AA, Poortman J, Nabuurs M, Thijssen JHH. Endogenous concentration and subcellular distribution of estrogens in normal and malignant human breast tissue. Cancer Res 1985; 45: 2900-6.
[63] Suba Z. Circulating and local estrogen concentrations are protective against breast cancer in obese women. In: Rahman A, Zaman K Eds. Topics in Anti-Cancer Research. Bentham Sci Pub, 2015; 4: 3-42.
[64] Bulun SE, Chen D, Moy I, Brooks DC, Zhao H. Aromatase, breast cancer and obesity: A complex interaction. Trend Endocrinol Metab 2012; 23(2): 83-9.
[65] Zhao Y, Agarwal VR, Mendelson CR, Simpson ER. Estrogen biosynthesis proximal to a breast tumor is stimulated by PGE2 via cyclic AMP, leading to activation of promoter II of the CYP19 (aromatase) gene. Endocrinology 1996; 137: 5739-42.
[66] Brueggemeier RW, Richards JA, Petrel TA. Aromatase and cyclooxygenases: Enzymes in breast cancer. J Steroid Biochem Mol Biol 2003; 86: 501-7.
[67] Suba Z, Kásler M. Interactions of insulin and estrogen in the regulation of cell proliferation and carcinogenesis. [In Hungarian]. Orv Hetil 2012; 153(4): 125-36.
[68] Suzuki T, Miki Y, Akahira J. I, Moriya T, Ohuchi N, Sasano H. Review: Aromatase in human breast carcinoma as a key regulator of intratumoral sex steroid concentrations. Endocrine J 2008; 55: 455-63.
[69] Sasano H, Miki Y, Nagasaki S, Suzuki T. In situ estrogen production and its regulation in human breast carcinoma: From endocrinology to intracrinology. Pathology International 2009; 59: 777-89.
[70] Tan J, Buache E, Chenard MP, Dali-Youcef N, Rio MC. Adipocyte is a non-trivial, dynamic partner of breast cancer cells. Int J Dev Biol 2011; 55(7-9): 851-9.
[71] Liu E, Samad F, Mueller BM. Local adipocytes enable estrogen-dependent breast cancer growth: Role of leptin and aromatase. Adipocyte 2013; 2(3): 165-9.
[72] Bollet MA, Savignoni A, De Koning L, Tran-Perennou C, Barbaroux C, Degeorges A, *et al*. Tumor aromatase expression as a prognostic factor for local control in young breast cancer patients after breast-conserving treatment. Breast Cancer Res 2009; 11(4): R54.
[73] Miller WR, O'Neill J. The importance of local estrogen synthesis in the breast. Steroids 1987; 50: 537-48.
[74] Iwase H, Yamamoto Y, Yamamoto-Ibusuki M, Murakami KI, Okumura Y, Tomita S, *et al.* Ethinylestradiol is beneficial for postmenopausal patients with heavily pre-treated metastatic breast cancer after prior aromatase inhibitor treatment: a prospective study. Br J Cancer 2013; 109(6): 1537-42.
[75] Jordan VC. The new biology of estrogen-induced apoptosis applied to treat and prevent breast cancer. Endocr Relat Cancer 2015; 22(1): R1-R31.
[76] Liu H, Lee ES, Gajdos C, Pearce ST, Chen B, Osipo C, *et al.* Apoptotic action of 17beta-estradiol in raloxifene-resistant MCF-7 cells *in vitro* and *in vivo*. J Natl Cancer Inst 2003; 95(21): 1586-97.
[77] Skoog L, Humla S, Axelsson M, Frost M, Norman A, Nordenskjöld B, *et al.* Estrogen receptor levels and survival of breast cancer patients. A study on patients participating in randomized trials of adjuvant therapy. Acta Oncol 1987; 26(2): 95-100.
[78] Kuske B, Naughton C, Moore K, Macleod KG, Miller WR, Clarke R, *et al.* Endocrine therapy resistance can be associated with high estrogen receptor alpha (ERalpha) expression and reduced ERalpha phosphorylation in breast cancer models. Endocr Relat Cancer 2006; 13(4): 1121-33.
[79] de Leeuw R, Neefjes J, Michalides R. A role for estrogen receptor phosphorylation in the resistance to tamoxifen. Int J Breast Cancer 2011; (2011) ID 232435.
[80] Jiang J, Sarwar N, Peston D, Kulinskaya E, Shousha S, Coombes RC, *et al*.Phosphorylation of estrogen receptor-alpha at Ser167 is indicative of longer disease-free and overall survival in breast cancer patients. Clin Cancer Res 2007; 13(19): 5769-76.
[81] Robinson DR, Wu YM, Vats P, Su F, Lonigro RJ, Cao X, *et al.* Activating ESR1 mutations in hormone-resistant metastatic breast cancer. Nat Genet 2013; 45(12): 1446-51.
[82] Shin A, Kang D, Nishio H, Lee MJ, Park SK, Kim SU, *et al.* Estrogen receptor alpha gene polymorphisms and breast cancer risk. Breast Cancer Res Treat 2003; 80: 127-31.
[83] Holst F, Stahl PR, Ruiz C, Hellwinkel O, Jehan Z, Wendland M, *et al.* Estrogen receptor alpha (ESR1) gene amplification is frequent in breast cancer. Nat Genet 2007; 39(5): 655-60.
[84] Broude, E., Roninson, I.B. Inhibitors of cdk8/19 for use in treating estrogen receptor positive breast cancer. US20160000787 (**2016**).
[85] Sukumar, S., Jin, K. Compositions and methods for treatment of tamoxifen resistant breast cancer. US9062308 (**2015**).
[86] Kufe, D.W., Kharbanda, S. Combination anti-estrogen receptor cancer therapy using muc1 peptides and chemotherapeutics. WO2014164395 (**2014**).
[87] Jenkins, P. Treatment of drug resistance in hormone-sensitive cancer. WO2014023791 (**2015**).
[88] Chinnaiyan, A.M., Robinson, D., Wu, Y.M. Systems and methods for determining a treatment course of action. WO2015057635 (**2015**).
[89] Sauter, G. Simon, R. Stahl, P. Holst, F. Al-Kuraya, K. Ruiz, C. Detection of ESR1 amplification in breast cancer. US8101352 (**2012**).
[90] Andersson, T. Restoration of estrogen receptor-(alpha) activity. US20110124574 (**2011**).
[91] Seymour CB, Mothersill C. Breast cancer causes and treatment: Where are we going wrong? Breast Cancer (Dove Med Press*)* 2013; 5: 111-9.
[92] O'Shaughnessy J. Extending survival with chemotherapy in metastatic breast cancer. Oncologist 2005; 10(Suppl 3): 20-9.
[93] Tran BH, Nguyen TA, Hwang BH, Vidar EN, Davis GB, Chan LS, *et al.* Risk factors associated with venous thromboembolism in 49,028 mastectomy patients. Breast 2013; 22(4): 444-8.
[94] Walker AJ, West J, Card TR, Crooks C, Kirwan CC, Grainge MJ. When are breast cancer patients at highest risk of venous thromboembolism? A cohort study using English health care data. Blood 2016; 127(7): 849-57.
[95] Darby SC, Ewertz M, McGale P, Bennet AM, Blom-Goldman U, Brønnum D, *et al.* Risk of ischemic heart disease in women after radiotherapy for breast cancer. N Engl J Med 2013; 368(11): 987-98.
[96] Grantzau T, Mellemkjaer L, Overgaard J. Second primary cancers after adjuvant radiotherapy in early breast cancer patients: A national population based study under the Danish Breast Cancer Cooperative Group (DBCG). Radiother Oncol 2013; 106(1): 42-49.
[97] Esserman L, Yau C. Rethinking the standard for ductal carcinoma *in situ* treatment. JAMA Oncol 2015; 1(7): 881-3.
[98] Ozanne EM, Shieh Y, Barnes J, Bouzan C, Hwang ES, Essreman LJ. Characterizing the impact of 25 years of DCIS treatment. Breast Cancer Res Treat 2011; 129(1): 165-73.
[99] Suba Z. One thought on "DCIS study amplifies questions and demand for answers" by Deak D. Bill of Health, 2015.
[100] Solin LJ, Gray R, Hughes LL, Wood WC, Lowen MA, Badve SS, *et al.* Surgical excision without radiation for ductal carcinoma *in situ* of the breast: 12-year results from the ecog-acrin e5194 study. J Clin Oncol 2015; 33(3): 3938-44.
[101] Suba Z. Light deficiency confers breast cancer risk by endocrine disorders. Recent Pat Anticancer Drug Discov 2012; 7(3): 337-44.
[102] Narod SA. Breast cancer prevention in the era of precision medicine. JNCI J Natl Cancer Inst 2015; 107(5): djv078
[103] Zhang XH, Giuliano M, Trivedi MV, Schiff R, Osborne CK. Metastasis dormancy in estrogen receptor-positive breast cancer. Clin Cancer Res 2013; 19: 6389
[104] Páez D, Labonte MJ, Bohanes P, Zhang W, Benhanim L, Ning Y*, et al.* Cancer dormancy: A model of early dissemination and late cancer recurrence. Clin Cancer Res 2011; 18(3): 645-53.