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Chapter VII

Failures and Controversies of the Antiestrogen Treatment of Breast Cancer

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Abstract

In postmenopausal women, estrogens have unquestionable preventive and curative effects against atherogenic cardiovascular lesions, osteoporosis and neurodegenerative diseases.

Moreover, recent studies on correlations between hormone replacement therapy and cancer risk could justify preventive, anticancer capacities of estrogen both on smoking associated and hormone related cancers.

Experimental developing of antiestrogen compounds aimed to inhibit the binding of presumably harmful, endogenous estrogen to its receptor system so as to achieve a regression of hormone-related cancers. However, antiestrogens proved to be ineffective in the majority of selected receptor positive breast cancer cases and produced severe side effects, such as vascular complications and cancer development at several sites. Failure of antiestrogen therapy was designated as "endocrine resistance" of tumors.

Therapeutic failures resulted in further search for effective drugs against hormone responsive tumors, designed as selective estrogen receptor modulators (SERMs). All these endocrine manipulations yielded fairly controversial results in breast cancer therapy, but the basic principle of estrogen carcinogenicity remained unquestionable. Moreover, a dangerous new trend is the recommendation of antiestrogens for cancer prevention at several sites.

Publications on the estrogen treatment of advanced breast cancer cases are regularly returning and the results are encouraging. Advantageous apoptotic effect of estrogen on advanced breast cancers was established as an adverse effect instead of proliferative activity.

Considering the newly introduced principle of the anticancer capacity of estrogen, the old-fashioned estrogen therapy in cancer treatment is to be applied and further developed.

Introduction

The idea of correlation between female sexual steroids and breast cancer risk had emerged more than 100 years ago based on empirical findings. Breast cancer was demonstrated to exhibit remission after ovariectomy in premenopausal women [1]. However, postmenopausal women occurred also frequently among breast cancer cases and this was strikingly explained by their abnormally elevated endogenous estrogen levels [2].

Hormone replacement therapy (HRT) against postmenopausal complaints of women became widespread in the past decades, especially in the economically developed countries [3]. Its use provided an excellent possibility to study the correlations between estrogen and breast cancer risk. Increasing prevalence of breast and endometrial cancers in Northern Europe and in the US seemed to be in close correlation with the popularity of HRT use. Results of subsequent studies in the past century suggested that breast, endometrial and ovarian cancers are hormonally induced tumors [4]. Both endogenous and exogenous elevated estrogen levels were presumed to play an important role in the development and progression of these cancers [5]. Carcinogenic capacity of estrogen has become a popular scientific field both for clinicians and researchers up to now. However, recent clinical studies revealed unexplained, beneficial anticancer effects of HRT use against oral, esophageal, gastric, colorectal, cervical, liver and lung cancer [6,7,8,9,10,11].

In the meantime, synthetic and natural estrogens were developed as therapeutic agents and their application has unique and indispensable possibilities in women, such as provocation of ovulation, and contraception in premenopausal cases. In postmenopausal women, estrogens have unquestionable preventive and curative effects against atherogenic cardiovascular lesions, osteoporosis and neurodegenerative diseases [12]. Moreover, recent studies on correlations between HRT and cancer risk could justify preventive, anticancer capacities of estrogen, even against the cancers of highly hormone responsive organs [13,14,15,16,17].

Protective effect of parity against breast cancer is well established [18]. In animal experiments pregnancy equivalent levels of estradiol were highly effective in the prevention of mammary, colorectal and kidney cancer induced by carcinogenic compounds [19,20,21].

Experimental development of antiestrogen compounds aimed to inhibit the binding of presumably harmful, endogenous estrogen to its receptor system so as to achieve a regression of hormone-related cancers. Tamoxifen was the first, clinically available antiestrogen proposed for cancer treatment [22]. However, antiestrogens proved to be ineffective in the majority of selected, estrogen receptor (ER) positive breast cancer cases and produced severe side effects, such as vascular complications and cancer development at several sites. Failure of antiestrogen therapy was designated as “endocrine resistance” of tumors [23].

Therapeutic failures resulted in further search for effective drugs against hormone responsive tumors, designed as selective estrogen receptor modulators (SERMs). These compounds were thought to be ideal to block the special signal transduction pathways of estrogen in cancer cells so as to achieve a tumor regression and to avoid side effects.

Aromatase inhibitors compose a further group of antiestrogen compounds, which blocks the enzyme converting androgens and other steroids to estrogen in ovaries and extraovarian tissues. Expectations to achieve a complete estrogen deprivation without harmful agonistic estrogen effects yielded further disappointment because of an unreliable inhibition of tumor growth and severe side effects [24].

All these endocrine manipulations yielded fairly controversial results in breast cancer therapy. Moreover, a dangerous new trend is the recommendation of antiestrogens for cancer prevention at several sites, in spite of their unreliable effect and severe, even life threatening side effects [12].

Estrogen treatment of tumors also emerged as breast cancer therapy in advanced cases. Publications on this therapeutic method applied in advanced breast cancer cases are regularly returning and they report on fairly encouraging results [25,26,27]. Many authors tried to explain the inverse, beneficial anticancer effect of the presumably carcinogenic estrogen. Advantageous apoptotic effects of high dose estrogen on advanced breast cancers were achieved in human therapy and it was evaluated as an adverse effect instead of proliferative activity [28,29].

This review will discuss the diverse molecular mechanisms of estrogen action and the controversial associations between estrogen effect and cancer initiation. Considering the newly introduced principle of the anticancer capacity of estrogen the therapeutic possibilities of antiestrogen and estrogen therapy in cancer treatment will also be analyzed.

Molecular Mechanisms of Estrogen Action

Role of estrogen and its receptors in gene regulation are thoroughly studied [30,31,32,33]. Review of literary data on the molecular mechanism of estrogen action suggests that estrogen orchestrates the gene regulation of cell proliferation with high safety [30,31,32]. Cross talk of estrogen receptors with other hormonal and growth factor signals, their agonistic and antagonistic interplay with other gene regulators suggest an indispensable role of estrogen in cell biology. However, estrogen deficiency may elicit a breakdown of the exquisite surveillance of gene regulation and results in disorders in cell proliferation beyond retrieval.

Gene regulation mechanisms affected by estrogen have a fairly wide range during pregnancy, embryonic growth, development and differentiation, and have a crucial role in the hormonal and metabolic processes both in males and females throughout the life. Estrogen, based on its pivotal role in gene regulation, has been accused of its carcinogenicity. However, as estrogen is a proven commander in chief in the regulation of endocrine functions, immune system, metabolism, and cell proliferation an extreme safeguarding is necessary to have domination over these highly responsible mechanisms.

Estrogen Receptors and Their Actions

In 1962 an estrogen binding receptor protein had been identified from rat uterus, which was supposed to mediate all actions of estrogen [34]. More than three decades later, in 1996

another isoform of estrogen receptor (ER) was discovered and named as ER-β [35], whereas the former ER was renamed as ER-α. These ER isoforms are the products of two distinct genes located on distinct chromosomes [36].

ERs belong to the steroid-thyroid hormone nuclear receptor superfamily [37]. The classic, genomic mechanism of ER action is that estrogen binding activates ERs in the nucleus and they act as transcriptional modulators by binding to specific sequences in the promoter region of target genes [32,38]. However, other signaling pathways that deviate from the classical model have been discovered, and recently it has been accepted that ERs regulate gene expression by a number of distinct mechanisms [32].

ERs can also regulate gene expression without direct binding to DNA, which occurs through interactions between receptor protein and other DNA-binding transcription factor proteins in the nucleus [32]. This interaction between ERs and transcription factors may result in either stimulation or inhibition in gene transcription processes [37]. Moreover, by means of some not clearly identified mechanisms, ERs may regulate the expression of genes that do not contain estrogen responsive elements (EREs).

Evidences are accumulating that estrogen action have also non-genomic, membrane associated signaling cascades [31]. Non-genomic actions may influence both the protein functions as a cytoplasmic network of coordination and the regulation of gene expression in the nucleus. Non-genomic actions are exerted by membrane associated ERs. Controversial concepts have been published concerning the nature of ERs located at the plasma membrane. Some authors have suggested that the non-genomic estrogen effects are mediated through a subpopulation of the classical two ER isoforms [39].

Finally, genomic and non-genomic pathways of estrogen receptor signaling converge on the target genes [32]. Several signal transduction pathways may connect the non-genomic estrogen actions to genomic responses. Many nuclear transcription factors are regulated through non-genomic protein kinase mediated phosphorylation, and these transcription factors may thus become targets for non-genomic estrogen actions. This complex signaling system provides an exquisitely safety control and plasticity of tissue responses to estrogen and also contributes to the divergence of tissue-specific actions [31].

ER-α is widely expressed throughout the female genital organs, such as in the uterus, vagina, ovaries and mammary glands, whereas it is also highly exhibited in the brain, bone, liver and cardiovascular system [40].

ER-β has been shown to be the dominant isoform in the prostate, testis, salivary glands, ovary, vascular endothelium, smooth muscle, certain neurons and in the cells of immune system [41]. Estrogen receptors in immune competent cells enable estrogen to regulate defense mechanisms against inflammatory processes, infections, shock and even malignant transformation.

Some tissues, such as mammary glands, uterus, brain, bone and cardiovascular system express both ER isoforms, with the predominance of one or the other [37,41]. All these sites are especially endangered in postmenopausal women with decreased ovarian hormone production. Incidence of ischemic attacks in the central nervous system, osteoporosis and dyslipidemic cardiovascular diseases is conspicuously increased in estrogen deficient female cases after menopause. In these diseases estrogen therapy has more or less beneficial effects, especially in the initial phases. Dangers of estrogen loss related to the breast and endometrium are newly recognized [13].

Presence of both alpha and beta ER isoforms was confirmed in adipocytes deriving from the subcutaneous and intra-abdominal location with evident predominance of ER-α [42]. Fatty tissue accumulation is fairly dependent on the equilibrium of sexual steroid levels. Estrogen tends to promote the gluteofemoral fat deposition [43], whereas estrogen loss and androgen excess is associated with abdominal obesity and strongly increases the risk of insulin resistance [44].

Postmenopausal aromatase activity and estrogen production in the adipose tissue have important role in many biological processes [45]. Recently, adipocytes have been regarded as endocrine cells. Fatty tissue is hormone dependent and in animal models hormones and cytokines produced by adipocytes have actions in remote organs, such as liver, muscle, bone, central nervous system and others [46]. Human adipose tissue and its secreted products have important role in the hormonal and metabolic processes. Hormonal regulation of human adipocytes may serve as a cross road among obesity, hypertension and insulin resistance [47].

ERs display similar DNA- and ligand binding properties in vitro, however, ER-β shows lower transcriptional activity than ER-α. Experimental results revealed that in the absence of estradiol both type of ERs interacted with estrogen responsive elements (EREs) in the nucleus similarly. However, estradiol application enhanced the ERE binding of ER-α but not that of ER-β. Thus, the interaction between ER-β and ERE seemed to be independent of estradiol and could be impaired by its amino terminus [48]. These findings provided additional explanation for differences between ER-α and ER-β actions. Moreover, these data also reveal that estrogen loss may yield thorough changes in gene expression mechanisms.

Summarizing the literary data, estradiol action depends on the ratio of receptor isoforms expressed in the given tissue. Moreover, in case of the coexpression of two receptors, interactions of their signaling pathways will variably modulate the transcription of target genes [33]. Great variations in tissular distribution and isoform predominance of ERs have been a source of intense debate among researchers. However, a widely accepted concept is that though ER-α and ER-β have distinct biological functions supposedly they act in a close interplay.

These overwhelming routes of estrogen effects and ER actions support that estrogen may use many different signaling pathways and will evoke distinct gene responses depending on the types of target cells and on the nature of temporary intra- and extracellular stimuli.

Interplay between Alpha and Beta Estrogen Receptor Isoforms in the Regulation of Cell Proliferation and of Apoptotic Cell Death

Mitogenic effect of estrogen is well known and thoroughly investigated, especially in context of cancer initiation and progression. Principle of the hormonal contribution of estrogen to carcinogenesis is based on its physiologic functions in gene regulation by means of its nuclear and plasma membrane receptors [49].

Estrogen receptor isoforms (ERα and ERβ) differently regulate epithelial cell proliferation and apoptotic cell death in normal tissues [50]. Different actions of ER-α and ER-β were observed on neuroblastoma cells transfected with either ER-α or ER-β. Activation

of ER-α caused an increase in length and number of neurites, whereas ER-β activation resulted only in neurite elongation [51].

Discovery of selective agonists for ER-α and ER-β brought new possibilities to research the specificity of estrogen receptors. In an epithelial cell line obtained from pregnant mouse mammary glands both ER-α and ER-β was expressed. It was observed that selective agonists for ER-α provoke cell proliferation and agonists for ER-β inhibit cell growth on this in vitro model [50]. These findings suggest that a functional equilibrium of these two receptor isoforms may supply excellent surveillance for cell growth, differentiation and proliferation.

Development of the three knockout mouse lines: estrogen receptor-α and β knockout (ER-αKO, ER-βKO) [52] and aromatase enzyme knockout (ARKO) mice provided new opportunities for understanding the individual actions of the two estrogen receptors and of estrogen itself. In vivo studies on these knockout mice have demonstrated that ER-α and ER-β may have distinct, even opposite effects. Development of distinct autoimmune diseases in different knockout mice revealed that ER-α and ER-β have specific functions in the maintenance of normal immune system [33,53,54].

In addition to the specific function of each receptor, interplay between these two receptor isoforms has been reported when they are coexpressed. ER-β may oppose ER-α-mediated activity in many systems, moreover, ER-α and ER-β together may elicit completely opposite responses in the presence of estradiol [38,55]. In ER-βKO mice, ER-α-mediated gene expression was increased by 85% as compared with animals expressing both receptors. This observation suggested that ER-β in normal mice reduces ER-α-regulated gene transcription, supporting the idea of a "ying yang" relationship between the two receptors [56].

However, considering the magnitude, complexity and safety of estrogen receptor signals, actions of ER-α and ER-β can be hardly imagined as a mechanical equilibrium of a seesaw. Though there are multiple cross talks and interplays between the two ER isoforms, both of them may have their autoregulation with a simultaneous feedback control of the actions of other one. ER knockout mice are artificially disabled animals and similar disorders may occur extremely rarely as spontaneous mutations. Unexpected, increased gene expression elicited by the singly remaining ER-α may probably be attributed to an emergency substitution of the action of missing ER-β isoform.

Signal transduction and cross talk of these receptors provide a safety, momentary equilibrium of cell growth and mitotic activity defined by tissue type, functional activity and environmental influences. Both receptors bind 17β-estradiol (E2) with similar affinity; however, they can activate certain promoters differentially [57]. Moreover, on some promoter ERβ can behave as an inhibitor of ERα activity [58], and being aware of their mutual regulatory capacity ERα is also capable to block ERβ.

Physiological resistance to E2 induced cell proliferation was observed in mouse mammary cell line, HC11, which reflects the situation of late pregnant and lactating state of mammary glands [59]. Although these cells expressed both estrogen receptors they showed no proliferative activity.

These findings underline that expression of any receptor is not equal with its proliferative impact. A suggested mechanism responsible for resistance to E2-induced proliferation may be the reduction in coactivators and increase in corepressors [50]. Interplay between the activities of ER isoforms may explain that a concomitant activation of ERα and ERβ may equalize the opposing cellular responses regarding proliferation and apoptosis.

Both ERα and ERβ selective ligands were administered independently and effects exerted by one or the other receptor isoform were studied. PPT, an ERα-selective agonist provoked an increase in cell number nearly 50%, while DPN, an ERβ selective ligand caused a decrease of 30%. In neuroblastoma cells ERα agonist increased cell division and ERβ agonist provoked cell growth [50]. Both selective ligands had positive effects on cell metabolism as measured by dehydrogenase activity. Activation of each receptor alone increased cell metabolism independently as either mitotic activity or cell growth requires increase in cellular metabolism. However, the activation of both receptors simultaneously, either by E2 or by PPT-DPN combination produced equilibrium without increased metabolic activity.

The proliferative effects of E2 are mediated not only by transcriptional activation from ERE promoters but also by interactions of ERs with AP-1 binding proteins via protein-protein interactions [60]. Nevertheless, the ER signal transduction pathways affecting the cell proliferation are highly and diversely regulated and the mechanistic assumption regarding the exclusively opposing activities of ERα and ERβ are not completely justifiable. Results of experimental studies applying either alpha or beta ER agonists do not reflect physiological processes.

Cell proliferation is not an isolated process being quite independent of metabolic, hormonal and immunologic regulation. Protein, lipid and carbohydrate synthesis, mitochondrial activation, cell growth and their control by hormonal signals (e.g. growth hormone) are the predecessors of normal mitotic activity [61]. Estrogen and ER signals are essential players to harmonize the regulation of all these cellular functions. Separated activation or blocking of each ER isoforms may produce thorough alterations and disturbances in these processes. For example, ERα-knockout mice show severe insulin resistance [50]. This finding suggests that isolated block of ERα activity may have inhibitory effect on cell division but provokes metabolic disturbances, which will increase both cancer initiation and progression. Similarly, apoptotic effect of isolated ERβ activation is associated with insulin resistance and oxidative stress.

Presumably, there are two main estrogen-activated pathways, that is, proliferation by ERα and apoptotic cell death by ERβ [50]. Activation of both pathways by estrogen may be responsible for a dynamic equilibrium of cell number. In a non-malignant cell line the ratio of ERα to ERβ expression in cells may determine whether estradiol will induce proliferation or not [50]. However, forced separation of the activity of two ER isoforms will cause a breakdown of regulation even in healthy tissues and further disturbances in tumors without beneficial anticancer effects.

Embryonic development justifies the necessity of all ambiguous actions of estrogen. High estrogen levels in pregnant women ensure both explosion-like cell proliferation and the cessation of mitotic activity.

To date there is no consensus on the role of ERβ in breast cancer development [62]. In ER-positive breast cancers ERα mRNA was shown to be upregulated, whereas ERβ was downregulated. Other studies analyzed ERα and ERβ protein expression in normal breast tissue and histopathologically distinct breast lesions of different prognostic risk [63]. Important role of tissue concentration, relative expression and interaction of these two ER isoforms was established in modulating mammary tumor genesis.

ERβ expression was significantly decreased in breast cancer samples and metastatic lymph node tissues as compared with healthy mammary tissues [64]. Loss of ERβ expression could be one of the events involved in the development of colon cancer [65]. ERβ specific

agonists might be candidates for treatment of some cancers [50,66]. Predominance of ERβ in highly differentiated tumors with preserved signal transduction pathways is a possibility for an induction of apoptotic cell death in estrogen-rich environment.

All these associations suggest that the overwhelming complexity of the transduction signal of estrogen receptors cannot be completely understood as our current knowledge probably covers only a small segment of their capacities. It is quite impossible to interfere with or even improve this highly controlled safeguard of estrogen on cellular mechanisms.

Considering the complex anticancer capacity of estrogen there is no sense to produce alternative compounds for the selective inhibition of its presumed "carcinogenic" activity with the preservation of beneficial estrogen effects.

Cross Talk between Receptor Signals of Estrogen and Growth Factors

Non-genomic actions of estrogen are too rapid to be attributed to RNA activation and protein synthesis. These actions are common properties of steroid hormones and are frequently associated with the activation of various protein-kinase cascades [67]. By these ways ERs have thorough surveillance on cell proliferation, which is their basic function during growth, differentiation and whole cell life.

Membrane associated ERs may activate membrane tyrosine kinase receptors in various cell types. ER-α activated by estradiol interacts directly with insulin-like growth factor-I (IGF-I) receptor, leading to its activation [68], and hence the activation of mitogen associated protein kinase (MAPK) signaling pathway [69]. These data demonstrate that estradiol activated ER-α is required for the rapid activation of IGF-I receptor signaling cascade.

Estradiol liganded ERs in the plasma membrane can rapidly stimulate signal transductions that are G-protein coupled. This was reported to occur through the transactivation of the epidermal growth factor receptor (EGFR) or insulin-like growth factor-I receptor (IGF-IR) [39]. Estrogen can overregulate the IGF-I gene transcription by involving an AP-I enhancer [68].

Estrogen signaling is coupled to growth factor signaling with a feedback mechanism directly impacting the function of growth factor receptors [30]. ER signaling pathways involving specific complexes of cytoplasmic proteins might either quench or augment the growth factor activities! On the other hand, the mitogen activated protein kinase system (MAPK) is capable of the phosphorylation of human estrogen receptor in vitro, and in cells treated with epidermal growth factor (EGF) and insulin like growth factor (IGF) in vivo. Thus the activity of the amino-terminal AF-l of ER may be modulated by the phosphorylation of Ser118 via the Ras-MAPK cascade of growth factor signaling pathways [70].

Estrogen dependent response in the uterus and mammary gland involves estradiol specific responses as well as responses resulting from a convergence of growth factor and ER-initiated activities [71,72].

Studies in rodents have shown that EGF is able to mimic the uterotropic effects of estrogen suggesting a physiological coupling of growth factor and steroid receptor signaling pathways. These results supplied a "cross-talk" model in which EGF receptor signaling resulted in activation of nuclear ERs [73]. However, in ER knockout mice epidermal growth

factor could not elicit estrogen-like response, which revealed that intact ERs are necessary for the activation of ER target genes by growth factors [74].

In the absence of ER-ligand, the activators of protein kinase A or inhibitors of protein phosphatases are able to stimulate ER-dependent gene expression. Both EGF and IGF-I may stimulate the transcriptional activity of ERs in an estrogen independent manner. Cross talk between ERs and membrane receptors for growth factors may have physiological role in an estrogen deficient environment [75].

Estrogen has a crucial role in the regulation of growth hormone receptor (GHR) activities as well [61]. Estrogen attenuates growth hormone (GH) action by means of inhibition of its secretion and suppression of cellular GHR functions. Estrogen and GH concentrations are strongly correlated during puberty and later, in adult life GH levels are regularly higher in women than in men. In man the stimulatory effect of androgens on GH secretion depends on the ratio of their prior aromatisation to estrogens. All these observations suggest that estrogens play a major and positive role in the regulation of GH-IGF-I axis in both genders [61], which may be in close correlation with their antidiabetogenic and anticancer capacities.

Estrogen Receptors and Human Cancers

There are literary data on the supposed roles of estrogen and membrane-associated ER signaling pathways in human cancer induction [76,77], particularly in breast cancer cases [78,79,80].

Interactions of estrogen and growth factor receptors have been recently revealed in breast, lung and cholangial cancers [81,82]. In the majority of in vitro experiments on different tumor cell lines a close synergism of expressed estrogen and growth factor receptors was supposed in the modulation of cell growth and proliferation.

In human breast epithelial cells both growth inhibition and growth stimulation by estradiol were observed depending on the rate of ERs and GFRs. Moreover, the expression of epidermal growth factor receptor and estrogen receptor is often inversely correlated in human breast cancer cells [83].

Estrogen treatment could inhibit lung carcinogenesis by reducing levels of IGF-I [84], which is a potent mitogenic agent for several malignancies, including lung cancer. These results suggest rather an alternative role of estrogen and growth factor actions on tumor cell proliferation.

Role of estrogens in the development of lung cancer has been suggested as ERs were demonstrated in non-small cell lung cancers. In recent studies a favorable prognosis for patients with non-small cell lung cancer was established, when tumor cells exhibited increased expression of ERβ [85,86]. By contrast, the absence of Erβ nuclear staining correlated with poor survival in both men and women with lung cancer [87]. Associations of increased ERβ expression with a better prognosis of non-small cell lung cancer suggest an anticancer capacity of estrogen signal and raise the idea of beneficial effect of estrogen administration. This assumption seems to be justified by a recent study, which established a significant correlation between hormone replacement therapy and reduced lung cancer risk in postmenopausal women [11].

Highly expressed epidermal growth factor receptors were demonstrated in a variety of solid tumors, including oral cancer, and their activity enhanced the tumor growth and invasive capacity [88]. However, the role of estrogen effect in oral cancer development is not clearly understood. Estrogen receptor expression in oral cancers showed no correlation either with tumor progression or the mortality of patients [89]. Further studies indicated that cross talk between ERs and EGFRs means a common contribution to the development and progression of squamous cell cancer in head and neck region [90]. Nevertheless the presumed synergistic carcinogenic capacity of ERs and EGFRs would be a constant danger for cancer initiation without contraregulatory impact.

Aromatase inhibitors, which lower the estrogen ligand for ER and pure ER antagonists, which destroy the receptor, are used to cure breast cancer. Recently, recognition of a dynamic, inverse relationship between the expression of ERs and EGFRs has brought more excitement [83]. In apparently ER-negative breast cancer cells, a potential restoring of ER expression emerged by inhibition of growth factor signaling to overcome resistance to endocrine therapy [91]. Abandonment of estrogen deprivation and antiestrogen therapy in breast cancer cases should be more advantageous against growth factors instead of the idea of combined antiestrogen and anti-growth factor therapy.

Inhibition of vascular endothelial growth factor (VEGF) expression by ER-α exhibited in HEClA endometrial cancer cell line [92]. This finding suggests that the inhibition of capillary proliferation in tumors may be an important part of the anticancer capacity of ERs.

Estrogen receptor signal is thoroughly defended from the changes of serum estrogen levels by compensatory feedback mechanisms even in tumors. This suggests that elevated estrogen level may hardly produce gene alterations. Excessive estradiol treatment decreased the ER protein quantity in MCF-7 human breast cancer cell line within a few hours [93]. Similarly, estrogen administration evoked a decrease in ER-α protein and estrogen responsiveness in rat pituitaries [94]. On the other hand, in experimental animals the low serum estrogen levels increased the mRNA level of ERs [95]. This strict feedback regulation suggests that ER overexpression in highly estrogen responsive cells means an increased estrogen demand induced by estrogen deficiency rather than defense against excessive hormone supply.

Estrogen and growth factors are potent mitogenic stimuli that share important competitive capacities in the control of cell proliferation. Cross talk between ER and growth factor signals assures finical equilibrium and may result in either synergistic or antagonistic effects on gene regulation. Exquisitely organized genomic and non-genomic actions of ERs and their convergence on the target genes rather serve as a defense of gene regulation with a safety surveillance of multitude players in cell biology than as risk for malignancy.

In the absence of estrogens, the biological equilibrium of gene regulation is endangered. There is no estrogen to exert its indispensable cellular actions and to oppose overwhelming growth factor activities. Accumulation of non-liganded ERs in estrogen deficient milieu may also result in emergency coupling of GF and ER signals resulting in a compensatory activation of nuclear ERs. Moreover, excessive IGF-I and EGF signals might modulate the functions of exposed, non-liganded ERs through their phosphorylation on certain residues [30]. These might be dangerous processes as unopposed growth factor activities and the altered phosphorylation of non liganded ERs may provoke a blunder in the gene regulation of cell proliferation. Breakdown in the main regulatory signals may result in cancer initiation [96].

Estrogen Synthesis in Tumors

Recently, postmenopausal estrogen production has been justified in the cells of different organs with high estrogen demand [33]. Aromatase activity and estrogen synthesis in the endothelium, glia, bone, breast and endometrium evoked many controversies and debates. Some authors regarded estrogen accumulation in the breast tissue of postmenopausal women as a justification of hormone induced carcinogenesis in spite of a decreased circulatory estrogen level [97]. However, this extraovarian estrogen synthesis may be a defensive mechanism for organs, which exhibit enhanced vulnerability in estrogen deficient environment rather than a possibility to access chronic diseases, even cancers in postmenopausal cases.

In situ synthesis of estrogens by breast cancer tissue provided potential explanation for high estradiol concentrations in the mammary neoplasms of postmenopausal women [98]. Moreover, in breast cancer cases a local estrogen production of carcinoma cells, stromal cells and adipocytes adjacent to the tumor was justified by abundantly expressed aromatase enzyme activity [99]. Intratumoral aromatase activity and increased in situ estrogen concentration were particularly demonstrated at the invasive front of cancers where the tumor-stromal interactions may define the spread of cancer cells [100].

Considering the concept of the anticancer capacity of estrogen, this hormone synthetizing interaction between cancer and stromal cells may be evaluated rather as a defense mechanism against tumor invasion at the hot zone. Estrogen synthesis by stromal cells and adipocytes adjacent to the invasive front of cancer may be regarded as a defensive host reaction against tumor spread.

On the contrary, the estrogen-producing capacity of highly differentiated breast cancer cells seems to be a kamikaze action. Taken into account the consequent ER positivism of highly differentiated, slowly growing breast tumors, they may preserve the aromatase activity and thus the estrogen synthetizing capacity of their mother tissue. Estrogen production of highly differentiated, ER-positive breast cancer cells may explain their slow tumor spread and the long lasting survival of patients with such tumors.

Antiestrogen and Estrogen in Cancer Therapy

Tamoxifen was originally designated as an antiestrogen compound, which selectively binds to estrogen receptors inhibiting their signal pathways. It is widely used as an adjuvant therapy for early stage ER positive breast cancers. However, the benefits of this antiestrogen compound are not equivocal in cancer therapy [24].

Even among women with ER positive breast cancers only 40-50% of patients exhibit primarily successful tumor regression [101]. These results suggest that more than half of ER positive breast cancers are de novo resistant to this endocrine therapy (102). A large proportion of earlier responsive breast cancers may acquire secondary resistance during tamoxifen therapy resulting in tumor progression and death [103]. Moreover, tamoxifen may elicit common side effects, which maybe occasionally life threatening [103,104,105,106]. Increased prevalence of stroke, pulmonary emboli and malignancies at several sites associated

with tamoxifen administration has been surprisingly attributed to its preserved estrogenic activity [23].

Explanation for this high rate of therapeutic failures designated as “resistance” may be that tamoxifen is regarded rather a selective estrogen receptor modulator (SERM) with variable agonistic and antagonistic activities. Its effects fairly depend on the distribution and ratio of ER-alpha and ER-beta expression in the tumors and on further factors influencing receptor binding [107]. In therapy resistant ER positive breast cancers tamoxifen is thought to behave like an estrogen similarly as it is presumed in bone, liver and coronary arteries, rather than an antiestrogen [108,109]. Taking tamoxifen for either SERM or antiestrogen, unsuccessful treatment on more than the half of selected patients with receptor positive breast cancer exactly defines its therapeutic failure.

Though there are overwhelming advances in understanding the signal pathways of ERs [132] their cross talks and interplays with epidermal growth factor receptors (EGFRs) and insulin-like growth factor receptors (IGFRs) hide many obscure details [110]. These correlations have undoubtedly essential role in the stimulation and inhibition of tumor growth. Being aware of the highly regulated ER signals even in differentiated tumors and supposing a mixture of agonistic and antagonistic impacts of SERMs it is impossible to prophecy, which signal pathways will be stimulated or inhibited.

Further group of antiestrogens is known as aromatase inhibitors, which prevent the activity of P450 aromatase [24]. This enzyme converts steroid precursors and androgens to estradiol both in healthy tissues and tumors and its inhibition means estrogen deprivation. Clinical investigations have demonstrated that aromatase inhibitors are at least as effective, or even better than tamoxifen. However, they have severe side effects related to estrogen depletion, such as osteoporosis and spontaneous bone fractures. By the introduction of aromatase inhibitors as therapeutic agents the supposed estrogen agonist activities of SERMs are successfully avoided but their anticancer effects are insufficient.

Being aware of the anticancer capacity of ER signal, estrogen deprivation caused by aromatase inhibitors seems to be less harmful as compared with tamoxifen, which destroys ERs.

Accidental tumor inhibitory effect of antiestrogens can be explained by several ways. In case of availability of intact ERs there are possibilities for getting estrogen signal over estrogen deprivation. Lower estrogen concentration may elicit a higher ER expression level by means of feedback mechanism, which may be a refugee for the endangered estrogen action [95]. Moreover, EGFR signal may also activate intact ERs by an accidental coupling of their signaling pathways so as to evoke an estrogen-like impact in emergency situation caused by estrogen deprivation [74]. By means of such complicated mechanisms estrogen signal makes its escape and may inhibit the tumor progression in spite of antiestrogen administration. Tamoxifen is a powerful inhibitor of mitochondrial electron transport chain and increase superoxide radical generation by its ER blocking activity [111], which are cytotoxic effects with possible anticancer capacity.

Results of widespread antiestrogen tumor treatment by SERMs seem to be ambiguous. These controversial data can be equally explained based on the principle of either the carcinogenic or anticancer capacity of estrogen. Considering the supposed carcinogenic capacity of estrogen, like Dr. Jekyll, tamoxifen may contribute to the tumor regression as a receptor antagonist. However, like Mr. Hyde, cloaked in the disguise of an agonist it is supposed to cause harmful tumor progression as well [101]. Recognition of the anticancer

capacity of estrogen helps to evaluate tamoxifen in breast cancer therapy quite inversely. ER agonistic impact associated with tamoxifen use may be regarded as getting estrogen signal through its inhibition, which results in beneficial tumor regression. However, if estrogen signal is defeated by the ER antagonistic actions of tamoxifen it might provoke local and metastatic spread of breast cancers.

Cancer provoking impacts of antiestrogens are equivocally justified by the exhibition of malignancies as their side effects, such as endometrial and gastrointestinal cancers [23]. Nevertheless, one can hardly imagine that the same compound has exclusive therapeutic action on breast cancer and in the meantime induces further malignancies at several sites by means of binding to the same receptor system. As a matter of fact, antiestrogen is not an adequate choice for breast cancer therapy either in theory or in practice.

Alternatively, estrogen therapy was also introduced as a possibility in breast cancer treatment with promising results. High dose estrogen was successfully administered as endocrine therapy for postmenopaUSl women with advanced breast cancer before the introduction of tamoxifen [25,27]. Subsequently, the use of estrogen as anticancer therapy was near completely abandoned. Recently, the "old-fashioned" high-dose estrogen treatment in patients with advanced breast cancer and previous estrogen deprivation was demonstrated to be effective in prospective clinical trials [27]. Moreover, women with advanced breast cancer and acquired resistance to aromatase inhibitor therapy were successfully treated even by lower doses of oral estradiol as well [112]. Low dose estrogen therapy resulted in a markedly lower incidence of serious adverse effects and at the same time good tumor regression.

Anti-tumor mechanism of estrogen therapy remained unknown. Nevertheless, estrogen may become an apoptotic trigger rather than a survival signal after many years of estrogen deprivation [29,112]. One can hypothesize that long-term aromatase inhibitor treatment may sensitize the hormone receptor positive breast cancer cells even to a low dose estrogen therapy.

Conclusion

Successful treatment of patients with advanced breast tumors by estrogen administration seems to justify the anticancer capacity of estrogen. Nevertheless, the ambiguous, unreliable therapeutic effects and high toxicity of antiestrogens suggests the necessity of finding new strategies in cancer treatment instead of fighting against a presumed endocrine "resistance".

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