Chapter 13

Selective Estrogen Modulators as an Anticancer Tool:
Mechanisms of Efficiency and Resistance

Surojeet Sengupta and V. Craig Jordan*

Abstract

The majority of breast cancers are estrogen receptor (ER) positive and depend on estrogen for growth. Therefore, blocking estrogen mediated actions remains the strategy of choice for the treatment and prevention of breast cancer. The selective estrogen receptor modulators (SERMs) are molecules that block estrogen action in breast cancer but can still potentially maintain the beneficial effects of estrogen in other tissues, such as bone and cardiovascular system. Tamoxifen, the prototypical drug of this class has been used extensively for the past 30 years to treat and prevent breast cancer. The target of drug action, ERs alpha and beta, are the two receptors which are responsible for the first step in estrogen and SERM action. The SERM binds to the ERs and confers a unique conformation to the complex. In a target site which expresses antiestrogenic actions, the conformation of the ER is distinctly different from estrogen bound ER. The complex recruits protein partners called corepressors to prevent the transcription of estrogen responsive genes. In contrast, at a predominantly estrogenic site coactivators for estrogen action are recruited. Unfortunately at an antiestrogenic site such as breast cancer, long term SERM therapy causes the development of acquired resistance. The breast and endometrial tumor cells selectively become SERM stimulated. Overexpression of receptor tyrosine kinases, HER-2, EGFR and IGFR and the signaling cascades following their activation are frequently involved in SERM resistant breast cancers. The aberrantly activated PI3K/AKT and MAPK pathways and their cross talk with the genomic components of the ER action are implicated in SERM resistance. Other down stream factors of HER-2 and EGFR signaling, such as PI3K/AKT, MAPK or mTOR pathways has also been found to be involved in resistance mechanisms. Blocking the actions of HER-2 and EGFR represent a rational strategy for treating SERM resistant phenotypes and may in fact restore the sensitivity to the SERMs. Another approach exploits the discovery that low dose estrogen will induce apoptosis in the SERM resistant breast cancers. Numerous clinical studies are addressing these issues.

Introduction

Selective estrogen receptor modulators (SERMs) are molecules which bind to estrogen receptors (ERs) and confer either estrogen-agonistic (estrogen-like) or estrogen-antagonistic (antiestrogen-like) actions in various estrogen target tissues and cells. In other words, the same SERM molecule can be estrogen agonistic in some tissues, as well as estrogen antagonistic in others, in the same organism at the same time. This pharmacology is unique and has allowed the SERMs

*Corresponding Author: V. Craig Jordan—Alfred G. Knudson Chair of Cancer Research, Fox Chase Cancer Center, 333 Cottman Avenue, Philadelphia, PA 19111-2497, USA. Email: v.craig.jordan@fccc.edu

Innovative Endocrinology of Cancer, edited by Lev M. Berstein and Richard J. Santen.

to be not only valuable tools to dissect the subcellular action of estrogen but also has opened the door to important therapeutic applications. However, SERMs did not appear suddenly as a new drug group but were originally referred to as nonsteroidal antiestrogens[1] that have continuously evolved and been evaluated for different clinical application during the past 50 years.

Nonsteroidal antiestrogens were originally investigated as agents to modulate reproductive functions.[2] They were effective as post coital contraceptives in rats[3] but actually induced ovulation in subfertile women.[4] The failure of antiestrogen to become antifertility agents throughout the 1960's resulted in a decline in interest by the pharmaceutical industry in developing the drug group. Nevertheless, the molecules were of pharmacological interest and became important tools in endocrine research to decipher the actions of estradiol (Fig. 1). As a drug group, the nonsteroidal antiestrogens were noted to block estrogen binding to its target tissues e.g., uterus, vagina and some breast cancers[5-7] because they were competitive inhibitors of estradiol binding to ER.[8,9]

One compound ICI 46,474 was studied extensively because fashions in research changed significantly during the 1970s. There was a new focus on cancer research which, in this case, built on the prior experience with reproductive endocrinology.[10] ICI 46,474, the failed contraceptive was reinvented to become tamoxifen (Fig. 1), the first antiestrogen for the treatment of breast cancer.[11] This in turn caused an evaluation of the molecular mechanisms of its antitumor action. During 1970s a treatment strategy was developed in the laboratory so that tamoxifen was subsequently targeted to the patients with ER positive tumors, administered as a long term adjuvant therapy in early stage disease which resulted in a significant advance in cancer therapy with survival advantages for hundreds of thousands of patients.[12]

In the laboratory, the discovery that tamoxifen needed to be hydroxylated to 4-hydroxytamoxifen to achieve high binding affinity for the ER[13,14] created an important laboratory tool to examine antitumor actions in vitro, to study structure function relationships[1,15] and ultimately to discover the actual molecular mechanisms of antiestrogen action at the ER level.[16] Overall the SERMs have played a pioneering role in cancer treatment both as laboratory tools and targeted agents in cancer

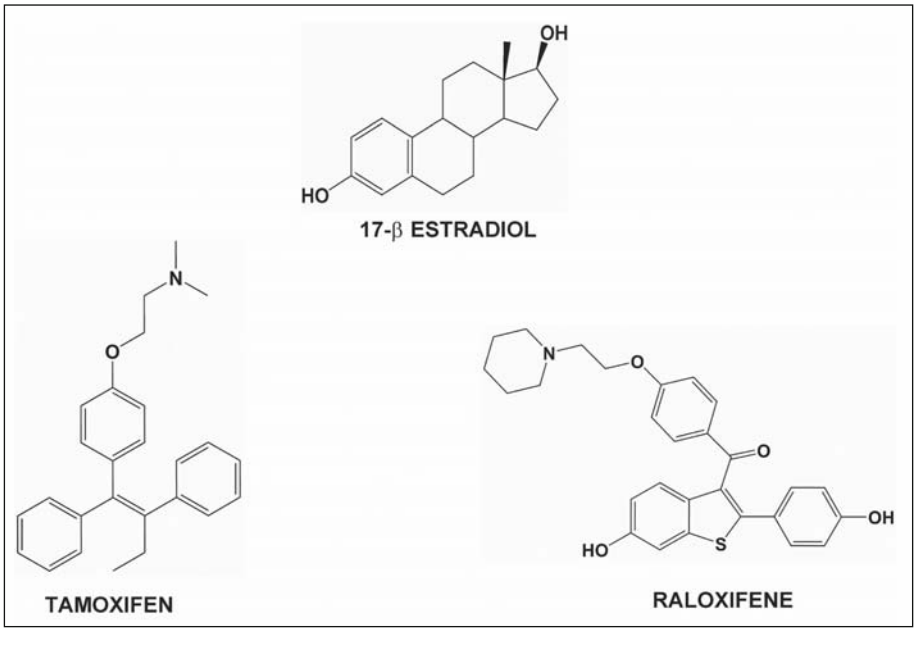

Figure 1. Chemical structures of 17-β estradiol, tamoxifen and raloxifene.

therapeutics. This chapter will trace their continuing development and current role in deciphering the complex signaling pathways that occur with the evolution of antihormonal drug resistance.

Estrogen, Tamoxifen and Cancer

As early as 1896, Dr. George Thomas Beatson noted that ablation of the ovarian stimulus (estrogen) restricted the growth of breast cancers.[17] Unfortunately only limited numbers of the breast cancer responded to the ablative surgery. More than 50 years later, the studies by Elwood Jensen,[18] that initially defined the target site specificity of estrogen action, helped further in understanding the requirement of the ER for the estrogen dependent growth of breast cancers.[19] The potential of tamoxifen (known as an anti-estrogen, at that time) to be used as an anti-breast cancer agent was recognized when it was reinvented from a failed contraceptive to become the first targeted drug for the treatment of breast cancer (see above).[11] Numerous studies using laboratory animals demonstrated the anti-tumor effects of tamoxifen. Early studies using a carcinogen-induced rat mammary tumor model revealed that tamoxifen was able to inhibit the growth as well as the tumor initiation.[20-24] However, long term therapy was stated to be the correct clinical strategy for the adjuvant treatment of breast cancer.[25,26] Similar findings were subsequently noted in xeno-transplanted ER positive breast cancer cells in the athymic (immuno-deficient) mice model. Tamoxifen was able to inhibit the estrogen-induced growth of the ER expressing breast tumors (MCF7 and ZR75) but not of ER negative (MDA-MB 231) tumors.[27,28] Overall these studies clearly indicated the anti-tumor effects of tamoxifen in ER positive breast cancers. The knowledge from the laboratory experiments, that tamoxifen could be used as a therapeutic agent to treat ER positive breast cancers, were successfully translated to clinical trials.[29,30] An early overview study combining 40 adjuvant tamoxifen trials noted highly significant benefits in both disease-free and overall survival.[31] A subsequent overview of randomized trials relevant to tamoxifen indicated that longer (5 years) duration treatments with tamoxifen are beneficial than shorter (1-2 years) treatments. Significant reduction in mortality was also observed with 5 years of treatment than shorter treatments.[12] Unfortunately treatment duration more than five years do not produce further benefits,[32] however, effective continuing reduction in breast cancer recurrence is noted for more than a decade after the termination of tamoxifen therapy.[12,33] The clinical trials for tamoxifen as an adjuvant therapy for breast cancer also revealed that 5 years of tamoxifen therapy reduces the recurrence of breast cancer and also the incidences of contralateral second primary breast tumors by fifty percent.[12,34] This led to the possibility that tamoxifen has potential as a chemo-preventive agent. However, the chemosuppresive actions of tamoxifen was already established earlier in experiments done in laboratory animals.[20,35] Several studies have now established that tamoxifen can significantly reduce the number of ER positive breast cancers in high risk group of both pre and post-menopausal women,[33,36-39] and is currently in use for therapeutic prevention of ER positive breast cancers in high risk population.

The idea that SERMs could be multifunctional medicines was based on the laboratory observations that a failed breast cancer drug keoxifene[40] (LY156758) actually maintained bone density in ovariectomized rats[41] and the same doses prevented mammary cancer in rats.[42] Most importantly, keoxifene was less estrogenic than tamoxifen in the rodent uterus[43] and was shown less active at stimulating human endometrial cancer growth in laboratory animals.[44] The publication of the idea[35,45] that nonsteroidal compounds of the same class as tamoxifen could be used to prevent osteoporosis in postmenopausal women but prevent breast cancer at the same time directly led to the subsequent re-examination of the pharmacology of keoxifene and the renaming of the compound into raloxifene (Fig. 1). The clinical investigation that a SERM could be used to prevent osteoporotic fractures but at the same time reduce the incidence of breast cancer[46] created a new dimension in chemoprevention.[47] Raloxifene was advanced for testing against the veteran tamoxifen to reduce breast cancer incidence in high risk postmenopausal women in the study of tamoxifen and raloxifene or STAR trial. Recent reports[48] demonstrate that raloxifene is equally effective as tamoxifen in preventing breast cancers in post-menopausal women. The study also showed lower incidence of endometrial cancer associated with raloxifene treatment than in case of tamoxifen.

Therefore, the clinical foundation to discover the ideal SERM has now been established. The SERM should prevent breast and endometrial cancer but increase bone density and reduce fractures. The challenge of molecular medicine for the future is to decipher the endocrine mediated control mechanisms for reversing or slowing the development of atherosclerosis, reducing hot flashes and defining the importance of estrogen regulated CNS function. To achieve these goals there is now a focused effort to understand the molecular modulation of estrogen action using SERMs as laboratory tools in estrogen target tissues and to understand SERM-stimulated drug resistance to optimize cancer control.

Molecular Mechanism of SERM Action

Mechanism of SERM action depends upon several factors. Essentially, SERMs bind to ERs α and/or β subtypes and confer a unique conformation to the ER. The complex further recruits coregulators and other accessory proteins at the estrogen-responsive elements of the promoters of specific genes to activate or repress transcription.[49] To completely understand the individual roles of these factors, we will discuss them separately.

Estrogen Receptors

Two sub-types of ERs α and β are responsible for the estrogen or SERM mediated effects. Different binding affinities of SERMs to these receptors and differential expression of these two sub-types in various target cells may account for selective modulation in some tissues.[50] In addition, hetero-dimerized ERs α and β may induce unique effects on estrogen- and tamoxifen-dependent gene expression.[51] A recent report also indicates that ER β mediates the effects on ER α induced transcription in ER positive breast cancer cells.[52]

Structurally, ER protein can be subdivided into six domains based on the function controlled by that region. The A/B domain contains one of the two transcriptional activation functions (AFs), known as AF1 which is largely involved in estrogen-independent activation of transcription. Another activation function domain, AF2, is located in the E domain which also harbors the ligand binding domain (LBD) and is involved in estrogen/ligand-dependent activation.[53] The structural studies of LBD of ERs α and β complexed with a SERM reveal that reorientation of the AF2 helix (helix 12) after the binding of the SERM to the hydrophobic pocket of the LBD and the interaction of amino acid asp351 of ERα with the alkylaminoethoxyphenyl side chain of tamoxifen are crucial for the corepressor recruitment to the surface of SERM-receptor complex.[16,54,55] Due to the usage of different mutants of ERα for the amino acid asp351 it is known that shielding and neutralization of asp351 by the side chain of raloxifene is critical in defining the antiestrogenicity of this SERM.[56] The involvement of the asp351 is further exemplified by changing the aspartate to glycine which abolishes the estrogen-agonist activity of tamoxifen, while retaining its antagonistic property.[57] AF2 region of the agonist-bound receptor is particularly important for the interactions of steroid receptor coactivators (SRCs 1-3) via the interacting amino acid motif LxxLL. Recruitment of these co-activator(s) to the promoters of estrogen responsive genes is also responsible for facilitating the activation of transcriptional machinery by chromatin remodeling. Additionally, SERMs may also show differential AF1 activity mediated by corepressor binding.[58] Using ERE-reporter constructs, it has been shown that AF1 domain of ERα is actively involved in agonist-induced gene expression whereas AF1 domain of ER β is involved very weakly.[59]

The activated ER binds to the specific estrogen responsive elements (ERE), found within the promoter region of responsive genes. Significantly, the nature of these DNA sequences also influences the recruitment of the coregulator proteins to the ER at the promoters. Using various ERE containing DNA sequences, it has been found that liganded ER α and β regulate the interaction of the coregulators depending upon the type of ERE, to which the receptor is bound.[60]

Coregulators

Interaction of particular coregulators (co-activators and corepressors) with the liganded estrogen receptors modulates the transcription of the responsive genes. Around 200 coactivators are currently known, which are associated with 48 nuclear receptors.[61] The coactivators undoubtedly

play defining roles in the activity of SERMs by cell or tissue specific expression pattern of genes. Studies have indicated that the relative abundance of a co-activator, SRC1 (steroid coactivator 1) in uterine cells is responsible for the agonistic activity of tamoxifen in those cells, whereas tamoxifen acts as an estrogen antagonist in breast cancer cells where the SRC1 levels are low.[62] However, raloxifene, another related SERM, does not recruit SRC-1 even in the uterine cells,[62] underscoring the fact that the SERM induced conformation of estrogen receptor is crucial for the interaction of coregulators. Consistent with these findings, earlier studies have reported tamoxifen-induced growth of endometrial cancer cells but not of breast cancer cells in athymic mice[63] and also that raloxifene (keoxifene) is less estrogenic to endometrial cancer cells.[44] These finding translate to clinical experience.[48] Furthermore, SERMs can also increase the stability of the co-activators (SRC1 and SRC3) and thereby enhance the transcriptional capability of other nuclear receptors.[64] In addition to transcriptional regulation, relative abundance and stability of co-activators, post-translational modifications particularly, different phosphorylation and sumoylation states of the co-activators can also drastically influence the capacity to interact with ER and other members of the transcriptional complex and regulate the gene activation.[65,66]

Corepressors proteins, on the other hand are functional counterparts of co-activators, which are associated with transcriptionally inactive promoters and thus help repress the expression of genes.[67] There are fewer corepressors known than the co-activators. In the case of ER, the co-repressors are known to interact with the unoccupied and antagonist bound receptor. Nuclear receptor corepressor (NCoR) and silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) are the two most extensively studied corepressors in connection with ER. The ER bound to raloxifene or 4-hydroxytamoxifen (a potent antagonist metabolite of tamoxifen) is known to recruit NCoR and SMRT to the promoters of estrogen responsive genes and repress transcription.[62,68,69] It has been shown that inhibition of NCoR or SMRT by using antibodies can enhance the agonistic property of 4-hydroxytamoxifen.[70] Moreover, using fibroblasts from NCoR null mice, 4-hydroxytamoxifen was shown to be relatively potent ERα agonist.[71] The critical role of NCoR and SMRT in 4-hydroxytamoxifen-induced arrest of cell proliferation of ERα positive breast cancer cells was illustrated when 4-hydroxytamoxifen-stimulated cell cycle progression was noted in the breast cancer cells deficient in NCoR and SMRT.[72] However this study also found that not all estrogen responsive genes were activated by 4-hydroxytamoxifen in NCoR and SMRT deficient cells, clearly indicating that other molecules may also be important in SERM-induced repression of estrogen responsive genes. Indeed, there are several other corepressor proteins known for ER. Metastasis associated protein 1 (MTA 1) is a corepressor found to mediate the ER transcriptional repression.[73] Another corepressor, known as repressor of estrogen action (REA) was able to potentiate the inhibitory effects of anti-estrogens including 4-hydroxytamoxifen. It was also found that REA interacted with ER and competed with the co-activator SRC1 for binding to the estrogen bound ER.[74,75] This again emphasizes the fact that the relative levels of coregulators may be important in deciding the outcome of the SERM action. The proteasomal regulation of NCoR is another factor which may influence the SERM action. Degradation of NCoR by 26S proteasome is known and is mediated by seven in absentia homologue 2 (Siah2).[76] Interestingly, estrogen mediated up-regulation of Siah2 in ER positive breast cancer cells has been implicated in proteasomal degradation of NCoR and subsequent de-repression of NCoR regulated genes.[77]

In addition to acting as a "transcriptional adapter" between the receptors and the transcriptional machinery, the coregulator itself or its complex possess various enzymatic activities such as acetylation, phosphorylation, methylation or deacetylation by which they are able to modify the local chromatin structure such as to make the environment conducive for gene expression or repression. Intrinsic histone acetyl transferase activity was found to be associated with co-activator SRC1 which helps in the activation of transcriptional expression.[78] In contrast, the 4-hydroxytamoxifen bound ER complex which recruits the corepressors NCoR and SMRT is associated with histone deacetylases and other chromatin modifying enzymes. The deacetylase activity promotes transcriptional repression.[62,79] Interestingly, another enzyme in the coactivator complex, CARM1 (coactivator associated arginine methyltransferase 1) has recently been implicated in modifying

the coactivator itself and inducing the degradation of the complex.[80] This suggests the ability of the enzymes in the complex to modify other proteins of its own complex apart from modification of the chromatin.

Evolution of SERM Resistant Breast Cancers

The preventive and therapeutic efficacy of SERMs for breast cancers is limited by the development of resistance for the SERMs. Initially, the development of SERM resistance was considered as overgrowth of ER negative cell population, over the growth arrested ER positive cells, by the antiestrogen (SERM) treatment.[81] However, we now know that there are various forms of SERM resistant breast cancer and studies of these resistant forms have led to novel therapeutic approaches. In general terms, SERM resistant breast cancers can be divided into two categories (a) de novo resistance and (b) acquired resistance. De novo resistance is defined as ER positive breast cancers which are nonresponsive to SERM therapy from the very beginning. De novo resistance can be demonstrated in the laboratory when ER positive MCF-7 breast cancer cells are stably transfected with the HER-2/neu gene. Tumors form very rapidly even during tamoxifen treatment.[82] Acquired resistance, on the other hand show those ER positive breast cancers which initially respond to SERM therapy, but do not continue to respond during long term therapy[81] (Fig. 2). This concept is illustrated in the laboratory if wild type MCF-7 breast cancer cells are inoculated into ovariectomized athymic mice and treated with tamoxifen. Initially most tumors do not grow but some tumors start to grow in the presence of the antiestrogen after about a year. If the growing tumors are transplanted into other athymic mice they will grow in response to either estrogen or tamoxifen.[83] Functional ER expression is still maintained in these SERM resistant cells. SERM resistance is unique because when the SERM is complexed with ER there is SERM stimulated growth. Examination of this form of SERM resistance in the clinic demonstrates that SERM resistant tumors can still respond to fulvestrant, a pure ER antagonist or the aromatase inhibitors which block the peripheral synthesis of estrogen in postmenopausal women.[84] This form of drug resistance i.e., SERM stimulated growth is referred to as phase I drug resistance (Fig. 2). Models for tamoxifen and raloxifene resistance are well described in the literature.[83,85]

Mechanism of SERM Resistance

Although the precise molecular mechanism for the SERM resistance is not completely understood, several genomic and extra-genomic factors are being shown to be involved in imparting resistance to SERMs or play a role in SERM induced growth of breast cancer cells. However, it is highly unlikely that any one particular mechanism is responsible for the SERM resistance in all patients. It could be possible that a combination of several factors may be responsible for the SERM resistance but for the sake of clarity these factors are discussed here individually.

Role of Epidermal Growth Factor Receptors (EGFRs) in SERM Resistant Breast Cancers

Signaling cascades originating from the cell surface of the cancer cells may drastically influence the genomic actions mediated by ER. One of the most prominent and well studied signaling pathway is the EGFR2, also known as HER-2/neu. HER-2, a receptor tyrosine kinase, is a member of the EGFR family and its amplification or overexpression is frequently associated with an aggressive phenotype of cancers.[86-88] Indeed, overexpressing HER-2 in ER positive MCF-7 breast cancer cells prevents the cells from responding to tamoxifen.[82,89] The mechanism by which HER-2 overexpression confers tamoxifen resistance and switches tamoxifen bound ER to an agonistic configuration has recently been described[90] (Fig. 3). An increased cross-talk between HER-2 and estrogen signaling pathways coupled with high SRC3 levels are responsible for subverting the ability of the tamoxifen bound ER to recruit corepressors. Instead the tamoxifen ER complex recruits coactivator SRC3.[90] Consistent with this conclusion, another study recently reported resensitization to tamoxifen by silencing the SRC3.[91] Additionally, in cells that overexpress HER-2, the agonistic activity of tamoxifen was reverted to an antagonist action by using inhibitors of HER-2

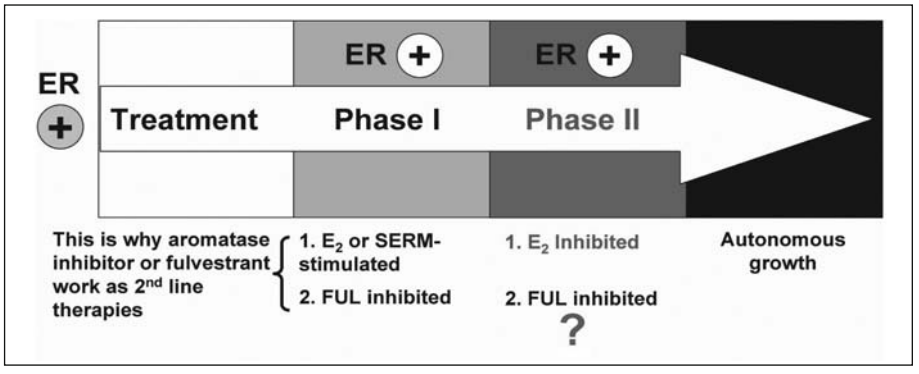

Figure 2. Diagram depicting different phases of SERM resistant breast cancers.

signaling.[82,90] This being the case, it is therefore important to understand the underlying mechanism of HER-2 initiated signaling cascades so that new therapeutic strategies can be formulated. Phosphatidylinositol-3-kinase (PI3K)/AKT and mitogen-activated protein kinases (MAPK) are the two critical signaling pathways which are activated aberrantly, in cells that overexpress HER-2.[92] Indeed, activation of AKT in ER positive breast cancer patients predicts decreased overall survival in tamoxifen treated patients.[93,94] Estrogen can rapidly activate AKT via the HER-2 pathway in cells expressing low levels of HER-2 and 4-hydroxytamoxifen can block this activation.[95] However, in breast cancer cells overexpressing HER-2, 4-hydroxytamoxifen can also activate AKT pathway in

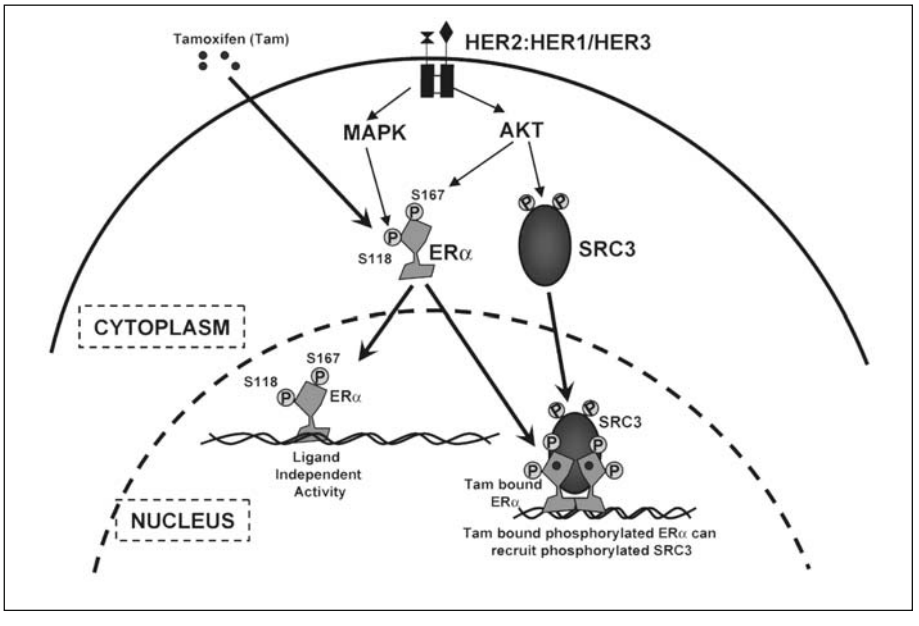

Figure 3. Schematic representation of cross talk between HER2 and estrogen signaling pathways. High HER2 expression activates AKT and MAPK pathways which can phosphorylate estrogen receptor (ER) and steroid coactivator 3 (SRC3). Phosphorylated ER can activate transcription independent of ligand. Tamoxifen bound phosphorylated ER can recruit phosphorylated SRC3 instead of corepressors and act as an estrogen agonist.

a HER-2 dependent manner,[90] exemplifying the conversion of 4-hydroxytamoxifen to an agonist. Both AKT and MAPK pathways can phosphorylate ER as well as the coactivator AIB1 (SRC3). Serine 167 residue of ER can be phosphorylated by AKT,[96] whereas serine 118 residue of ER can be phosphorylated by the MAPK pathway, both resulting in ligand-independent activation of estrogen receptor.[97,98] Not surprisingly, breast cancers with high levels of SRC3 along with HER-2 over-expression are associated with worse outcome following tamoxifen therapy, indicating resistance.[99] A recent study have also reported that specific phosphorylation of ER can modify the binding ability of ligands and also modulate its capacity to interact with co-activators.[100] In addition to HER-2, elevated level of EGFR/HER-1, another member of the EGFR family, is also correlated with poor prognosis and has been implicated in SERM resistant breast cancers.[101,102] Different members of EGFR family can dimerize, autophosphorylate and activate different signaling pathways. Long term treatment with tamoxifen, resulting in resistance, is also associated with increased translocation of ER α out of the nucleus and enhanced interaction with EGFR.[103] Similarly, high levels of HER-2 were found to increase the relocalization of ER α from nucleus to cytoplasm.[104] It is therefore evident from these findings that aberrant signaling cascades initiated by over-expressing EGFR and HER-2, particularly involving PI3K/AKT and MAPK pathways, are critically involved in cross talk with the genomic components of ER responses. All of these events may merge to create resistance to SERM treatment.

Other Factors Involved in SERM Resistant Breast Cancers

In addition to aberrant activation of AKT and MAPK pathways in SERM resistant breast cancers, several other factors have also been reported. The mammalian target of rapamycin (mTOR), which is a downstream target of PI3K/AKT and MAPK pathway,[105,106] is found to be involved in estrogen induced proliferation of ER positive breast cancer cells.[107,108] Furthermore, specific inhibitors of the mTOR pathway restore sensitivity to tamoxifen in a tamoxifen resistant cell line, both in vitro and in vivo.[109]

Another downstream target of EGFR and HER-2, is c-Src which phosphorylates p27 and impairs its inhibitory action on cyclin dependent kinase 2 (Cdk2) resulting in increased mitogenic activity. This mechanism is also implicated in tamoxifen resistance, as inhibition of c-Src was found to restore tamoxifen sensitivity.[110]

A rather novel approach to reversing tamoxifen resistance is to use disulfide benzamide (DIBA) that disrupts the zinc fingers of ER DNA binding domain and prevents the association of coactivators with 4-hydroxytamoxifen bound ER. DIBA was able to restore the tamoxifen sensitivity in several different tamoxifen resistant cells. However, this effect was achieved without altering the phosphorylation statuses of HER-2, MAPK, AKT and AIB1 in these cells.[111] It is possible that the use of DIBA with an inhibitor of phosphorylation would be a reasonable strategy for long term therapeutic use.

Therapeutic Options for SERM Resistant Breast Cancers

Since EGFR and HER-2 mediated signaling events play important roles in SERM resistant phenotype of breast cancers, blocking these pathways represent a logical approach in combating SERM resistance. Indeed, several laboratory studies have used selective inhibitors of HER-2 and/or EGFR in SERM resistant cells and reported beneficial outcomes, including reversal of SERM resistance.[90] A recent study[112] demonstrates that using a combination of three drugs, all targeting the HER2 by different mechanisms, along with tamoxifen or estrogen deprivation could effectively block the growth of HER2 overexpressing ER positive breast cancer in athymic mice. In another study using raloxifene resistant breast cancer cells, blocking of HER-2 activation by trastuzumab (humanized monoclonal antibody against HER-2) was found to decrease the growth of the resistant tumors in laboratory animals.[85] This approach was particularly effective in preventing the growth of tamoxifen stimulated endometrial cancers.[113] Clinical efforts are therefore directed towards using either small molecule inhibitors against EGFR and HER-2 or humanized monoclonal antibody against HER-2 as a monotherapy or in combination with other therapies

including SERMs, in patients not responding to endocrine therapies.[114] As mentioned earlier aromatase inhibitors or fulvestrant are equally effective at treating breast cancer patients who are already resistant to tamoxifen. However, laboratory studies[115] now show that the initial inhibition of tumor growth, by either fulvestrant or estrogen deprivation is quickly followed by resistance and all the resistant tumors exhibit elevated levels of phosphorylated AKT and MAPK.[115] Tumor control by fulvestrant or estrogen deprivation is enhanced when this approach is combined with therapy that inhibits the EGFR/HER-2 signaling. These findings further underscore the idea that inhibiting the downstream targets of AKT and MAPK pathway, like mTOR, may be of significant importance in attenuation of SERM resistance.[109]

Resistance to Long Term Antihormone Therapy

The laboratory models and mechanisms discussed so far really represent the early stages of drug resistance to SERMs. The models replicate treatment of metastatic breast cancer with tamoxifen and do not replicate the strategy of long term adjuvant therapy with 5 years of tamoxifen. To address this deficiency tamoxifen-stimulated breast tumors have been repeatedly transplanted into tamoxifen-treated athymic mice to replicate micrometastases that grow in a tamoxifen environment for years. Remarkably, the signal transduction pathways in tumor cells become reconfigured so that estrogen is no longer a survival signal but triggers apoptosis in phase II resistant breast cancer cells[116-118] (Fig. 2).

Estrogen Induced Apoptosis

Phase II tamoxifen stimulated tumors are dependent upon tamoxifen for growth and are cross resistant with raloxifene.[119] Indeed the converse is also true. Raloxifene-resistant breast cancer cells can be grown into tumors in athymic mice by treatment with either raloxifene or tamoxifen.[118] However, it is the dramatic antitumor effect of estrogen as a major factor in breast tumor cell survival that is intriguing. High dose estrogen therapy was originally used as a palliative treatment for postmenopausal metastatic breast cancer before tamoxifen, an antiestrogen, was developed during the 1970's.[11] Alexander Haddow[120] reported that high doses of synthetic estrogens would produce a 30% response rate in unselected patients and the responses would last about one year. Despite the fact that treatment with high dose estrogen therapy has slipped into disuse with the ubiquitous use of tamoxifen and new aromatase inhibitors, recent laboratory studies indicate that low dose, rather than high dose, estrogen could again find a place in the treatment paradigm of metastatic breast cancer. The first indication that this was true occurred when the findings that physiologic level of circulating estradiol could cause tumor regression in long term tamoxifen resistant tumors (phase II).[116,117] The idea is now being advanced to the clinic as there is every reason to believe that the concept will translate as a treatment for antihormone resistant breast cancer. It is already known that high dose estrogen produces a 30% response rate in patients whose tumors are refractory following exhaustive antihormonal therapy.[121]

Additionally the paradoxical effect of estrogen to induce apoptosis is not limited to SERM resistant breast cancer cells, but has also been observed in estrogen deprived breast cancer cells.[122,123] Although the precise mechanism of estrogen induced apoptosis is under intense investigation, studies have indicated the involvement of mitochondrial pathway of apoptosis in estrogen deprived cells,[124] and a different mechanism in raloxifene resistant cells.[118] Most importantly, laboratory studies have shown that the breast cancer cells that become resistant to estrogen induced apoptosis regain the sensitivity for SERM therapy.[117] Therefore, it is possible that cyclical treatments with SERM and estrogen may help to control breast cancer growth for a prolonged period.[125]

Conclusion

Currently, tamoxifen, the prototypical SERM, can be used to treat all stages of ER positive breast cancers and for chemoprevention in high risk women. The effectiveness of this class of drugs is based on selectively blocking the estrogen mediated effects in the breast cancer. The fact that the ER is such an important target and that majority of breast tumors are ER positive has made ER

blockade such a significant therapeutic success. This clinical success has led to the development of other SERMs in the group, like raloxifene, with fewer undesirable effects. However, despite significant advances the use of long term SERM treatment is ultimately associated with acquired breast cancer resistance. Nevertheless, studies during the past decade have identified specific signaling pathways that are involved in the cross talk with ER signaling, thereby creating resistance to SERMs. Although encouraging results and strategies are being developed to employ inhibitors of phosphorylation pathways it may be that the tumors develop too many signaling options to use a single approach to block resistance. In this regard the novel finding that estrogen will eventually induce apoptosis in SERM resistant breast cancer cells merits further detailed study for its wider therapeutic use. It may be that the skill of the ER to activate apoptosis can be used to identify an apoptotic trigger to kill cancer cells selectively.

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