Selective estrogen receptor modulation: Concept and consequences in cancer

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Extended exposure to the selective estrogen receptor modulators (SERMs) such as raloxifene to prevent osteoporosis and tamoxifen or the aromatase inhibitors to treat or prevent breast cancer are established therapeutic strategies. However, there are now clearly defined consequences of exhaustive antihormonal therapy in breast cancer. Ultimately, drug resistance to SERMs and aromatase inhibitors enhances cancer cell survival but a paradoxical supersensitivity to estrogen action develops that causes cancer cell apoptosis. The future exploitation of these novel data will allow selective killing of cancer with fewer side effects for patients.

Introduction

Estrogen mediates a broad spectrum of physiologic functions ranging from regulation of the menstrual cycle and reproduction to the modulation of bone density and cholesterol transport. The case for estrogen supplementation following menopause was based on the clinical observations that elderly women without circulating sex steroids had a higher incidence of osteoporotic fractures, coronary heart disease (CHD) and, most importantly for quality of life, hot flashes and night sweats. Conjugated equine estrogen alone was supplemented with medroxyprogesterone acetate to reduce the risk of endometrial cancer in postmenopausal women, and the combination is referred to as hormone replacement therapy (HRT). A regimen of HRT is effective in reducing osteoporotic fractures and is indispensable in treating severe menopausal symptoms (WGWHII, 2002). However, recent prospective clinical trials demonstrate that long-term HRT, i.e., 5 years or more, provides no overall benefit for women's health (MWSC, 2003; WGWHII, 2002). Although there are reductions in the incidence of colon cancer, osteoporotic fractures, and menopausal symptoms, there are increases in breast cancer, Alzheimer's disease, strokes, and blood clots (Figure 1; Chlebowski et al., 2003; MWSC, 2003; Shumaker et al., 2003; WGWHII, 2002). These definitive clinical studies have highlighted the opportunities for innovation in the selective modulation of estrogen target tissues (Figure 1).

Estrogen action at target sites around the body is mediated through related but distinct estrogen receptors (ERs) designated ERα and β (Enmark and Gustafsson, 1999). Estrogens bind to the ligand binding domain of the ER to induce a conformational change in protein structure that permits the subsequent dimerization and interaction with coactivator molecules (Figure 2; McDonnell and Norris, 2002; McKenna et al., 1999). The sequential activation of genes occurs through multiple mechanisms either directly at estrogen response elements in the promoter region of estrogen-responsive genes or through a tethering protein-protein interaction with cfos/jun B (AP-1) sites or Sp1 sites (Figure 2). These cellular signal transduction pathways can potentially be exploited to amplify tissue response selectivity. Alternatively, survival pathways in cancer could evolve to alter the entire responsiveness to ER signaling.

Traditionally, the science of pharmacology plays a critical role in drug discovery by using a receptor target to identify

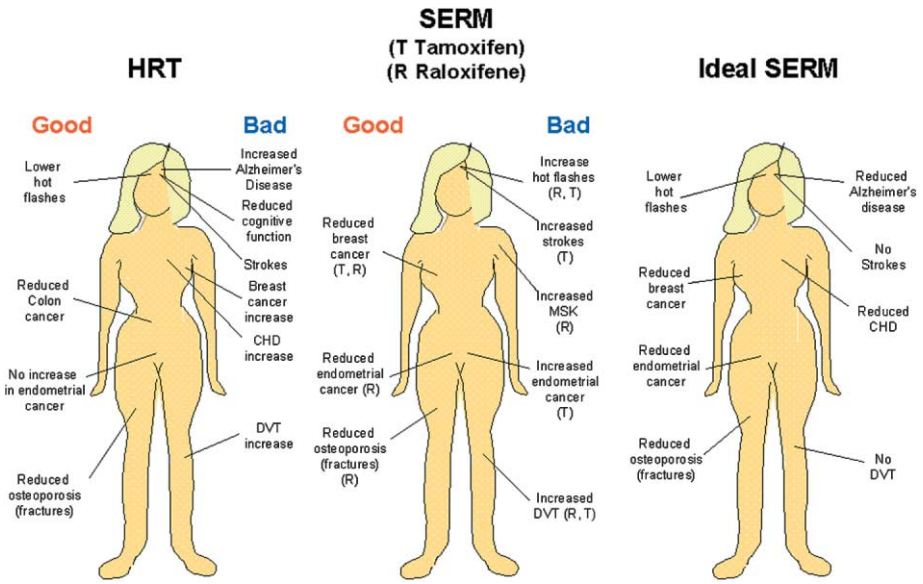

Figure 1. Progress toward an ideal SERM

The overall good or bad aspects of administering hormone replacement therapy to postmenopausal women compared with the observed site-specific actions of the selective estrogen receptor modulators tamoxifen and raloxifene. The known beneficial or negative actions of selective estrogen receptor modulators (SERMs) have opened the door for drug discovery to create the ideal SERM or targeted SERMs to either improve quality of life or prevent diseases associated with aging in women.

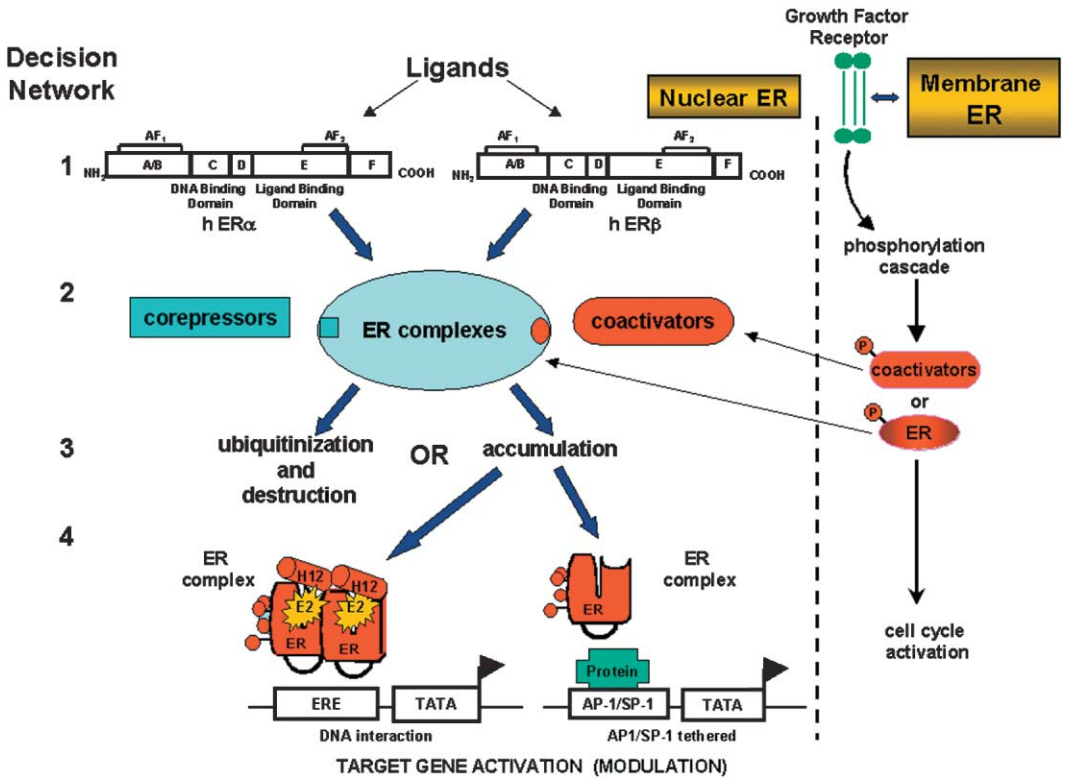

Figure 2. Complexity of SERM signal transduction

The decision network for estrogen or SERM action binding to nuclear estrogen receptor (ER) α or β receptor or membrane ER (decision 1). Receptor-specific or mixed specificity ligands bind to the ligand binding domain (E region) of the ERs to cause a ligand-specific perturbation in the receptor complex that creates opportunities for the complex to bind either coactivators or corepressors on the external surface (decision 2). The interactive proteins shunt the ER complex into transcriptionally active or inactive states. Although the expanding family of coregulators are being defined, this does not exclude the possibility of other interactive proteins could alter gene transcription through phosphorylation activation. This could be initiated rapidly either by membrane ER or constitutively through cell surface growth factor receptors. The next decision point (3) is where the complex or coregulators are ubiquitated and destroyed by the proteasome or accumulate to become promiscuous estrogen-like complexes. Again, phosphorylation may play an important role in the activity of the ER complex. The decision (4) to interact with the machinery involved with gene transcription can shunt the signaling pathway from positive or negative regulation based upon the ER concerned, the ligand, or whether there is a direct interaction with an estrogen response element (ERE) or a tethered interaction to proteins at AP-1 or SP-1 sites. Overall, the decision network creates a complex regulatory system at target tissues or in cancer where a growth advantage can be exploited in response to antiestrogen therapies.

select molecules for testing in the clinic. However, the recognition of the target tissues concept of selective estrogen receptor modulation by compounds originally referred to as nonsteroidal antiestrogens (Jordan, 1984) was noted first in laboratory animals and then successfully translated to the clinic (Jordan, 2001).

The clinical application of the SERM concept

The recognition of the SERM concept is an example of translational research that changed medical practice. Although the targeting of the ER with the nonsteroidal antiestrogen tamoxifen has increased selective survivorship in breast cancer (Jensen and Jordan, 2003), the strategic application of long-term antihormonal treatments (Jordan and Allen, 1980) has created an important increase in disease-free and overall survival (EBCTCG, 1998; Goss et al., 2003). However, tamoxifen is not a complete or pure antiestrogen, and the drug exhibits partial estrogen-like actions that could produce a suboptimal blockade of estrogen-stimulated breast tumor growth. Currently, aromatase inhibitors to produce an estrogen-free environment are demonstrating superiority to tamoxifen in controlling the growth of ER-positive breast cancer (ATAC Trialists' Group, 2002). Most importantly, the use of aromatase inhibitors for the treatment of breast cancer avoids some of the estrogen-like side effects observed in patients treated with tamoxifen. Tamoxifen is a partial estrogen agonists in the rodent uterus. Laboratory studies subsequently demonstrated that tamoxifen had the potential to stimulate growth of endometrial cancer but inhibit the growth of breast cancer (Gottardis et al., 1988). These data translated to a low but significant increase in the incidence of endometrial cancer in postmenopausal women treated with tamoxifen (Fisher et al., 1994; Fornander et al., 1989). However, the incidence of endometrial cancer is reduced during treatment with an aromatase inhibitor (ATAC Trialists' Group, 2002).

Clearly, the fact that tamoxifen increases the incidence of endometrial cancer is a significant concern for the application of tamoxifen as a chemopreventive for breast cancer in high-risk women. Nevertheless, the possibility that an antiestrogen could increase the risk for osteoporosis in well women was initially of greater concern for women's health in the 1980s. Tamoxifen maintains bone density in ovariectomized rats (Jordan et al., 1987; Turner et al., 1987), and this result translated to maintain-

ing bone density in postmenopausal patients (Love et al., 1992) with a nonsignificant reduction in fractures in a chemoprevention trial (Fisher et al., 1998). Thus, women with an increased risk for breast cancer treated with tamoxifen can anticipate a 50% reduction in the incidence of breast cancer (antiestrogenic) but a reduction of osteoporotic fractures (estrogenic) and an increase in the side effects of blood clots and endometrial polyps and cancer (estrogenic) (Figure 1; Fisher et al., 1998). This spectrum of SERM action creates a requirement for an intervention focused only on very high-risk women and a requirement for new SERM discovery programs.

However, there is difficulty in identifying target populations in breast cancer. Clearly, a broader strategy was required to enhance the potential of SERMs in women's health to prevent breast cancer. The approach that was taken was to exploit the potential of SERMs to reduce osteoporotic fractures but with the beneficial side effect of reducing the incidence of breast cancer (Lerner and Jordan, 1990). The result is raloxifene, originally a discarded breast cancer drug named keoxifene. Raloxifene (keoxifene) maintains bone density in ovariectomized rats (Jordan et al., 1987) and prevents carcinogen-induced rat mammary carcinogenesis (Gottardis and Jordan, 1987). These data subsequently translated to the clinic where raloxifene is effective at reducing osteoporotic fractures in women at risk (Ettinger et al., 1999) with a reduction by 70% in the incidence of breast cancer (Cummings et al., 1999). Raloxifene is currently available for the prevention of osteoporosis but with breast and endometrial safety. Raloxifene is also being evaluated for the ability to reduce the incidence of coronary heart disease (Mosca et al., 2001).

There is considerable interest in developing new SERMs as multifunctional agents in women's health (Jordan, 2003a, 2003b). However, the approach for the future will be based on the molecular modulation of emerging mechanisms rather than what happened in the past with the reinvention of nonsteroidal antiestrogens as receptor-targeted therapeutics from their original application as modulators of fertility (Jordan, 2003c).

Mechanisms of SERM action

The interpretation of a novel SERM at a target site involves a complex series of decision points that could shunt the receptor complex in one direction or another (Figure 2). The challenge is first to document fully the machinery available at target sites and then to understand the subcellular network of outcome opportunities. At present our basic understanding of the process is fragmentary, but current knowledge provides a reasonable basis for evaluating future targeted therapeutics (Figure 2).

The target site distribution of ERα and ERβ and differential ligand specificity and pharmacology (Enmark and Gustafsson, 1999) have created opportunities to develop receptor-specific ligands based primarily on differences in receptor affinity (Meyers et al., 2001; Stauffer et al., 2000). It is possible to envision the development of an ERα-specific antagonist to prevent breast cancer or an ERβ-specific agonist to enhance CNS functions or prevent colon cancer. However, the process of drug development based on receptor screening may be confounded by the complexities of the subsequent signal transduction pathways (Figure 2).

Considerable progress has been made during the past 5 years in understanding the molecular perturbations that occur in the ligand binding domain of ERα and β when complexed with a SERM (Brzozowski et al., 1997; Pike et al., 2001; Shiau et al., 1998). The essential structural determinant of the SERM molecule is a correctly positioned alkylaminoethoxyphenyl side chain that interacts with asp351 in ERα to modulate antiestrogenic action through corepressor binding to the external surface of the SERM receptor complex (Brzozowski et al., 1997; Shiau et al., 1998). The interaction of the SERM side chain with asp351 allosterically modulates the estrogenic and antiestrogenic action of tamoxifen and raloxifene. The tamoxifen ERα complex is much more promiscuous and estrogen-like than the raloxifene ERα complex, but estrogen and antiestrogen actions can be modulated by mutating asp351 (Liu et al., 2002; MacGregor Schafer et al., 2000). The interpretation of molecular studies could go some way to explaining the enhanced estrogen-like actions of tamoxifen in the uterus compared with raloxifene (Figure 1; Cummings et al., 1999; Fisher et al., 1998). Nevertheless, recent experimented evidence suggests that there is another dimension involved in the estrogen-like action of SERMS.

The relative concentration of members of the coactivator family (SRC-1, -2, or -3) or corepressors may regulate the response of a tissue to ERα. One possibility to explain target site specificity for SERM action would be to have site-specific coactivator interactions. Shang and Brown (2002) demonstrated, in one uterine cell line, that elevated SRC-1 enhanced the estrogen-like actions of 4-hydroxytamoxifen but not raloxifene. This effect was not noted in breast cancer cells.

Ultimately, the response of a tissue to a ligand-receptor complex will depend not only on the efficacy but also the concentration of receptor complexes available to interact with the gene regulatory machinery. This consideration draws into the equation the dimension of receptor complex destruction. The higher the level of low-efficacy complexes, the higher the probability of estrogen action. However, the efficacy and concentration of the activated ligand receptor complex is regulated not only by sensitivity to ubiquitization of ER (Wijayaratne and McDonnell, 2001) and subsequent destruction; the amount of coactivator proteins (Lonard et al., 2004) is also important to amplify or suppress the activation of a complex.

SERMs increase the levels of SRC-1 and -3 and also enhance the transcriptional activity of nuclear receptors other than ER in SERM-treated cells (Lonard et al., 2004). These events create additional opportunities for understanding the complexity of target site specificity with SERMs. Indeed, tamoxifen-induced increases in SRC-3 have previously been shown to occur through the indirect action of SERM-induced transforming growth factor β (Lauritsen et al., 2002). However, the complex preparations for gene transcription or protein activation are not the final decision the SERM or estrogen must make. There appear to be numerous additional pathways that can modulate the individual cells in a target tissue. The simplistic view that the ER complex activates genes through interaction with an ERE in the promoter region has evolved dramatically over the past decade. It seems that the promoter region can influence the shape of the ER complex, which in turn can alter the external shape of an ER complex and, as a result, coactivator or corepressor binding (Hall et al., 2002). Select genes could be sequentially regulated by the changing conformation of an ER complex being modulated by promoter interactions.

It is now recognized that the SERM ER complex is extremely promiscuous and can also activate genes through AP-1 (Webb et al., 1995) and SP-1 (Khan et al., 2003) (Figure 2) pro-

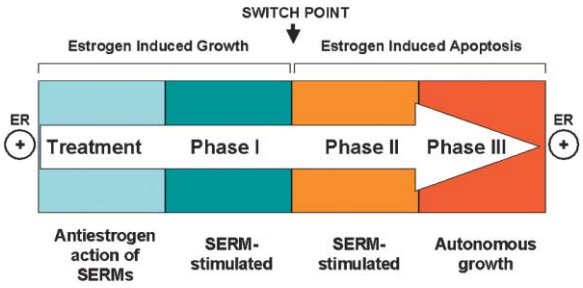

Figure 3. The evolution of drug resistance to SERMs

Acquired resistance occurs during long-term treatment with a SERM and is evidenced by SERM-stimulated breast tumor growth. Tumors also continue to exploit estrogen for growth when the SERM is stopped, so a dual signal transduction process develops. The aromatase inhibitors prevent tumor growth in SERM-resistant disease and fulvestrant that destroys the ER is also effective. This phase of drug resistance is referred to as Phase I resistance. Continued exposure to a SERM results in continued SERM-stimulated growth, but eventually autonomous growth (Phase III) occurs that is unresponsive to fulvestrant or aromatase inhibitors. The event that distinguishes Phase I from Phase II acquired resistance is a remarkable switching mechanism that now causes apoptosis, rather than growth, with physiologic levels of estrogen. These distinct phases of laboratory drug resistance (Lewis et al., 2004; Yao et al., 2000) have their clinical parallels and this new knowledge is being integrated into the treatment plan.

tein-protein interactions, and cell survival cascades may also be modulated by ER located in the cell membrane (Razandi et al., 1999). Most importantly, the bidirectional signaling between cell surface receptors (insulin-like growth factor and epidermal growth factor receptors) and ER will have profound effects on estrogen and SERM signaling opportunities (Levin, 2003). These membrane pathways can rapidly activate both ER and coactivators to enhance cell replication.

Overall, normal cells and tissues have the potential to be modulated by SERMs through a diverse and complex network of decision pathways. Understanding the potential targets will enhance the chances of novel designer SERMs to regulate or modulate numerous physiologic conditions. However, unlike the normal cell, the cancer cell adapts and evolves through selection in a changing drug environment. Understanding drug resistance to SERMs now creates new opportunities to exploit emerging discoveries in cancer cell regulatory pathways.

The evolution of drug resistance to SERMs

Twenty years ago, the development of drug resistance to antihormonal therapy in breast cancer was viewed as the insensitive ER-negative cells overgrowing ER-positive cells that were in growth arrest from antiestrogen treatment. Today, the conversation between the laboratory and the clinic has advanced therapeutics by recognizing various forms of drug resistance to tamoxifen. Current research is targeting resistance mechanisms to develop new therapeutic strategies. Resistance can be classified as either intrinsic resistance, where ER-positive breast cancer is initially refractory to antiestrogen treatment, or ER-positive disease that initially responds to antihormonal treatment but acquired resistance occurs subsequently. Acquired resistance can be caused by alterations in the ER signal transduction pathway converting the inhibitory SERM ERα complex to a growth stimulatory signal. Recent clinical studies (Osborne et al., 2003) indicate that tamoxifen is unlikely to be an effective therapy in ER-positive breast cancer patients who also have high levels of SRC-3 and HER2/neu. The cell surface signaling pathway can enhance phosphorylation of both the ER and SRC-3 (Font de Mora and Brown, 2000). Thus, the multiple opportunities to initially (intrinsic resistance) or eventually (acquired resistance) subvert the inhibitory actions of the tamoxifen ER complex creates a complex survival system for the cancer cell. This insight into the tumor options of either estrogen or tamoxifen-stimulated growth has resulted in improvements in therapeutics with either aromatase inhibitors that create a "no-estrogen" environment (ATAC Trialists' Group, 2002) or the pure antiestrogen fulvestrant (ICI 182,780) that destroys the ER (Wijayaratne and McDonnell, 2001). Both drug types are valuable for the treatment of tamoxifen-resistant breast cancer (Robertson et al., 2003).

However, current understanding of drug resistance to SERMs or estrogen deprivation is based on short-term (1–2 years) treatment periods. This treatment strategy was appropriate 25 years ago when the focus was on treating advanced disease, but today all trends are toward a decade of treatment in breast cancer (Goss et al., 2003) or indefinite treatment with raloxifene for the prevention of osteoporosis. Recently, the

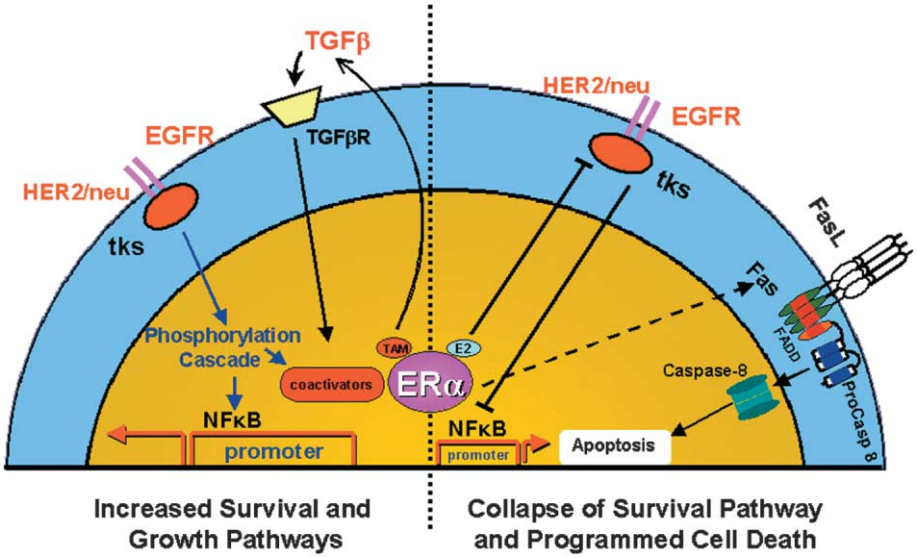

Figure 4. Life and death of Phase II SERM resistance

Putative mechanisms of estradiol (E_2)-induced apoptosis that occurs after the switch point in Phase II and Phase III SERM resistance. Drug resistance to SERMs occurs when the ER survival signal transduction pathway is blocked. Surviving cancer cells create enhanced cell surface signaling mechanisms (HER2/neu, EGFR) that initiate phosphorylation cascades that enhance the activity of the SERM ER complex either directly or indirectly through transforming growth factor β (TGFβ) and inducing coactivators that are phosphorylated. Long-term SERM exposure creates sophisticated, yet vulnerable, survival pathways that can be collapsed rapidly by estradiol with a loss of HER2/neu signaling and loss of prosurvival NFκB. The events that herald apoptosis occur in parallel during estradiol treatment. The death receptor fas is translated and a cascade of caspase activation condenses the chromatin and destroys the cell.

description of models of extended antihormonal therapy now provide new opportunities for reusing the ER as a novel therapeutic target in cancer (Figure 3).

The repeated transplantation of MCF-7 tamoxifen-resistant breast tumors into successive generations of tamoxifen-treated athymic mice or culture of MCF-7 cells under estrogen-free conditions with or without raloxifene results in the alteration of the signal transduction pathways for estrogen (Liu et al., 2003; Yao et al., 2000). Although estrogen is considered to be a survival hormone with the ability to initiate replication, drug resistance to estrogen deprivation occurs by developing cells with enhanced survival pathways that maintain the growth advantage for cancer cells. For example, cell surface signaling through HER2/neu is regulated by estrogen: without estrogen, HER2/neu mRNA is increased (Newman et al., 2000).

Exhaustive antiendocrine therapy causes the ultimate form of drug resistance, spontaneous growth (Figure 3). However, studies in the laboratory (Yao et al., 2000) and preliminary clinical studies (Lonning et al., 2001) demonstrate that estrogen, rather than acting as a growth stimulus, acts as an apoptotic agent through an ER-mediated mechanism in Phase II and Phase III resistant disease (Figure 3).

Clearly, there is potential to incorporate an "estrogen purge" into the long-term clinical treatment program. Laboratory studies already demonstrate that tumors that recur after estrogen-induced apoptosis are again sensitive to the antitumor actions of tamoxifen or estrogen withdrawal (aromatase inhibitor) (Yao et al., 2000). A strategy of cyclical antihormone treatment and estrogen purges may maintain patients with breast cancer for decades.

Molecular mechanisms of estrogen-induced apoptosis

Preliminary subcellular studies have identified the fas/fas ligand pathway as a putative mediator of estrogen-induced apoptosis in both long-term estrogen-deprived cells (a model of aromatase inhibition) (Song et al., 2001) and either tamoxifen- or raloxifene-resistant breast cancer cells (Liu et al., 2003; Osipo et al., 2003). The cancer cell survival pathways mediated by the HER2/neu cell surface signaling mechanisms collapse and so does the nuclear NFκB transcription mechanism. In parallel, estrogen induces the fas receptor (Liu et al., 2003; Osipo et al., 2003) that may herald apoptosis (Figure 4).

Overall, these studies provide an insight into the balance of cell survival and apoptosis that occurs through the ER. However, the unanticipated result that the pure antiestrogen fulvestrant blocks the estrogen-induced apoptotic pathway and enhances robust tumor growth by maintaining survival pathways (Osipo et al., 2003) illustrates the delicate balance between survival and cell death governed by the ER. A similar phenomenon occurs in the long-term estrogen-deprived cell line MCF-7:5C (Lewis et al., 2004). Estrogen induces rapid apoptosis in vitro and in vivo when autonomously growing cells are transplanted into athymic mice. However, the combined effect of the antiestrogen fulvestrant alone and the apoptotic effect of estrogen alone results in maximal growth of MCF-7:5C cells when both estrogen and fulvestrant are incubated together (unpublished data). It is also possible to provoke estrogen-independent growth in another breast cancer cell line T47D stably transfected with the cDNA for PKC α. Tumors grow spontaneously in athymic mice, but again estrogen rapidly causes tumor regressions through apoptosis (Chisamore et al., 2001).

Overall, it seems that a new general principle is emerging where the creation of an enhanced survival network in the cancer cell can be rapidly destroyed by the use of estrogen targeted to the ER. Discovery of the cellular survival mechanisms that subvert the central role of the ER in breast cancer may provide new advances in targeted therapies. Currently, the observation that half of the ER-positive breast cancers are responsive to antihormones could be viewed as an opportunity to restrict survival selectively with novel tyrosine kinase inhibitors and then activate the ER with either traditional or low-dose estrogen. The ER could also be used as the bait to discover a novel apoptotic target to exploit in future drug discovery.

Summary of SERM prospects

The successful therapeutic application of antihormonal strategies with tamoxifen and aromatase inhibitors has probably reached its zenith in the clinic, but study of drug resistance has now opened a new chapter in targeting cancer. There is currently a separation of objectives, with the aromatase inhibitors being used predominantly to treat breast cancer and the SERMs providing therapeutic opportunities as safer "hormone replacement" therapies to prevent osteoporosis and reduce breast and endometrial cancer (Figure 1). Nevertheless, extended or perhaps indefinite treatment regimes are now possible if late-phase antihormonally resistant disease can be destroyed with a short estrogen purge. Additionally, there are practical opportunities to broaden the value of the ER as a therapeutic target by devising logical treatment strategies for the patient with an ER-positive tumor that is refractory to antihormonal treatment. Although these new treatment options could potentially benefit patients, it is the potential of the ER to identify a novel apoptotic target that could dramatically advance selectivity in molecular therapeutics.

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