Selective Estrogen-Receptor Modulators and Antihormonal Resistance in Breast Cancer

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ABSTRACT

Selective estrogen-receptor (ER) modulators (SERMs) are synthetic nonsteroidal compounds that switch on and switch off target sites throughout the body. Tamoxifen, the pioneering SERM, blocks estrogen action by binding to the ER in breast cancers. Tamoxifen has been used ubiquitously in clinical practice during the last 30 years for the treatment of breast cancer and is currently available to reduce the risk of breast cancer in high-risk women. Raloxifene maintains bone density (estrogen-like effect) in postmenopausal osteoporotic women, but at the same time reduces the incidence of breast cancer in both high- and low-risk (osteoporotic) postmenopausal women. Unlike tamoxifen, raloxifene does not increase the incidence of endometrial cancer. Clearly, the simple ER model of estrogen action can no longer be used to explain SERM action at different sites around the body. Instead, a new model has evolved on the basis of the discovery of protein partners that modulate estrogen action at distinct target sites. Coactivators are the principal players that assemble a complex of functional proteins around the ligand ER complex to initiate transcription of a target gene at its promoter site. A promiscuous SERM ER complex creates a stimulatory signal in growth factor receptor–rich breast or endometrial cancer cells. These events cause drug-resistant, SERM-stimulated growth. The sometimes surprising pharmacology of SERMs has resulted in a growing interest in the development of new selective medicines for other members of the nuclear receptor superfamily. This will allow the precise treatment of diseases that was previously considered impossible.

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INTRODUCTION

The estrogen receptor (ER) is the trigger1 that initiates estrogen action in its target tissues (eg, uterus, vagina, and pituitary gland). The subsequent identification of the ER in some breast cancers created a mechanistic link to explain the hormonal dependence of some breast cancers.2 Ultimately, this knowledge was used to reinvent a failed postcoital contraceptive, ICI 464743 as tamoxifen, the first selective estrogenic properties of tamoxifen and antiestrogens at different target sites around the body, created a new dimension in drug development and enhanced therapeutic possibilities. The selective estrogenic properties of tamoxifen and raloxifene maintained bone density4 but the selective antiestrogenic properties prevented mammary carcinogenesis.5 These laboratory data were used to develop an evidence-based therapeutic strategy6,7 that has now become a clinical reality with the development of raloxifene. This second-generation selective ER modulator (SERM) prevents osteoporosis but also prevents breast cancer as a beneficial side effect.8 With this significant advance in therapeutics, it has become clear that the action of SERMs at different target sites can no longer be explained by an ER model that simply turns estrogen action on or off. Other physiologic factors must be involved.

In this article, we will describe our evolving understanding of SERM action at its target sites. Although the ER complex is programmed by the shape of the SERM buried inside the receptor, it is the new protein players called coactivators and corepressors9 that are now known to modulate and control the dynamics of the complex as it turns on or turns off subcellular signaling networks at target sites around the body. However, we believe it is important to state at the outset that although we have, by necessity, chosen to explain the molecular mechanism of SERMs to retain therapeutic relevance in oncology, we prefer to use the term steroid
receptor modulators (SRMs) when considering mechanisms. The molecular biology of selective activity is clearly universal within the steroid receptor superfamily. This fact has important therapeutic implications for future drug discovery.

**MECHANISMS OF SELECTIVE RECEPTOR MODULATOR ACTION**

Of the 48 members of the nuclear receptor (NR) family, approximately half have been determined to be regulatable by ligands. The remaining molecules are regulated by signaling pathways that impart post-translational modifications to these endocrine/metabolic transcription factors. The nuclear receptors are signal-dependent transcription factors that have two main purposes: (1) to locate target genes by binding at specific DNA sequences (termed hormone response elements [HREs]) that are located at these genes; and then, (2) to recruit transcriptional coregulators to the gene. Ligands can induce both activation and repression of target genes. NRs recruit coactivators to activate genes, and corepressors to repress genes. These two functionally different classes of molecules comprise the totality of 285-member coregulator superfamily, most of which are coactivators. The general domain structure of coactivators is shown schematically in Figure 1, and a great deal of additional basic and clinical information is provided on the Nuclear Receptor Signaling Atlas Web site (www.nursa.org). Although the NR coregulators were identified only approximately 11 years ago, they are generally accepted as the rate-limiting components of transcriptional control in mammals.

The molecular mechanisms by which distinct ligands can bind to the same nuclear receptor and yet exert tissue-specific actions, has been somewhat of a mystery until the last decade, when the contributions of basic receptor research have led to an enlightened viewpoint. We now realize the complexities and the relative importance of the fundamental elements that factor into the equations for tissue-selective SRM actions. These elements are (1) receptor isoform subtypes; (2) ligand-induced conformations of the receptor; (3) precise sequence compositions of the HREs; (4) nuclear receptor coregulators (coactivators and corepressors), which are recruited by the active or inactive conformation of the receptor to the gene site; and (5) cell and signaling context. Although the coregulator recruitment is of paramount importance, under most conditions, all five of the preceding events can have a modulating influence on the actions of an SRM.

![Diagram](A) The structure of the known estrogen receptors (ERs) with the identified (red) activating functions (AFs) that bind coactivators. Also identified is the DNA-binding domain (DBD) and the ligand binding domain. (B) A typical domain structure of a nuclear receptor (NR) coactivator is shown. There are two main domains: (1) a protein-protein interacting domain that binds other coactivators in the functional high molecular weight coactivator complex and (2) an enzymatic domain that either has intrinsic enzyme activity or binds a protein that has enzyme activity. Numerous enzyme activities have been demonstrated in the many coactivators discovered to date.
Multiple function-specific isoforms have been discovered for a number of receptors, including those for progesterone receptor (PR; PRα, PRβ), ER (ERα, ERβ), and glucocorticoid receptor (GR; GRα, GRβ).15 These isoforms have different primary structures and therefore beget different gene functions. Since the tissue concentration of receptor isoforms can vary in a tissue-specific manner, the functions of the cognate receptor ligand in a given tissue can vary also. Perhaps the ERα and the ERβ isoforms have the most contradictory functions, with ERα having a growth promoting action and ERβ having a growth-inhibitory action in certain tissues.20 Consequently, the tissue-selective ratio of ERα/ERβ can provide a tissue-selective function.

**LIGAND-INDUCED RECEPTOR CONFORMATION**

For many years it was suspected that a transcription-inducing ligand acted simply by shifting the equilibrium of its cognate NR from an inactive to an active conformation. Two complimentary experimental approaches helped to clarify receptor-mediated modulations. A comprehensive pharmacologic evaluation of the structure function relationship of estrogens and antiestrogens both at an ER-regulated prolactin gene target21,22 and by regulating breast cancer cell replication,23 built up a hypothetical model of molecular modulation. The pharmacologic studies concluded the size and position of the “antiestrogenic” side chain of the then nonsteroidal antiestrogens controlled the folding of the ER at an antiestrogenic region of the ER.21,24,25 Simply stated, the “crocodile” model proposed equilibrium mixtures of receptor jaws closed (estrogenic complex) or propped open by the ligand (partial estrogenic/antiestrogenic complex) to modulate gene function at target sites.16,27 Complementary early biochemical studies utilized protease structural mapping and antibody epitope mapping techniques to demonstrate that progesterone and estrogen bound to their cognate receptors and induced a conformational alteration in the carboxy-terminal tail of the receptor, whereby the tail flipped back over the ligand pocket and the active form was stabilized.28,29 It was the eventual x-ray crystallography of these molecules, however, that provided a more detailed picture of this model, whereby a c-terminal helix 12 was the lid that covered the ligand pocket and formed a landing platform for newly recruited coactivators (or corepressors).30-32 The newly recruited coregulators then carry out all of the reactions required for the entire transcriptional process (discussed further herein). Different receptors binding to the same genetic sequence can recruit different coactivators and thereby provide quantitatively or qualitatively different gene responses (Fig 2). Similarly, different ligands occupying the same receptor at a gene site can induce different structural conformations in that receptor and lead to recruitment of different coactivators, and consequently, different gene expression patterns.

**DNA BINDING ELEMENT (HRE) OF THE TARGET GENE**

The precise composition of different genomic HREs in mammals varies. HREs are usually composed of short inverted or direct repeats of approximately 7 deoxynucleotides each. When minor variations in the receptor contact sequence occur, and in combination with other surrounding transcription factors, the receptor can be forced into an altered conformation that in turn recruits different coregulators and provides distinct functions for these genes, if they are expressed in that tissue.33 This basic principle has been demonstrated, but it is unclear as to how often this is a significant factor in SRM actions. What is clear is that recruitment of the receptor complex to the HRE is cyclical with binding and destruction.34

**NUCLEAR RECEPTOR COREGULATORS**

Current opinions place the coregulators in the driving seat of tissue-specific actions of SRMs. The potency and selectivity for all subreactions of transcription reside in these coregulators, and thus, they are critically important for not only gene function, but also tissue-selective gene function. Currently there are approximately
285 NR coregulators, of which the vast majority are coactivators (approximately 40 are corepressor according to the Nuclear Receptor Signaling Atlas). Most occur in the majority of tissues, but at different individual concentrations in each tissue. Consequently, each tissue has a “quantitative fingerprint” of coactivators based on the relative concentrations of each molecule in that tissue. This inherited complement of coregulators provides a basis for tissue-selective actions by a given NR.

Coregulators function as large, high-molecular weight complexes of approximately six to seven coactivator proteins. Most of the coregulators are enzymes that participate in remodeling the local chromatin structure at the target promoter, initiating transcription by RNA polymerase, encouraging efficient elongation of RNA chain synthesis, regulating alternative RNA splicing, and, finally, destroying the active transcription factors at the promoter site. These series of substeps of transcription occur in rapid sequence (approximately 15 seconds apart) and are controlled by sequential occupation of the promoter by specific coregulator complexes that direct the transcriptional substep reactions.

For the most part, the coregulators are themselves regulated at the post-transcriptional level. Their intracellular concentrations are determined by their proteasomal degradation rates. Levels are raised by inhibiting the rate of degradation, and vice versa for lowering levels. Traditional ubiquitin-mediated degradation occurs, as well as an ubiquitin-independent turnover by 11S cap proteins such as REγ. Degradation can be inhibited by post-translational modification of a coactivator at certain sites; alternatively, specific kinases can phosphorylate these sites to promote higher cellular levels of coactivator.

The cell context plays a role in selective gene responses to ligand because differentiation produces cells with specific available gene complements for expression. The cell also has a predetermined basal concentration of each of the coregulators and their cognate activating/inactivating enzymes, thereby establishing a threshold of available regulatory molecules. This cellular concentration of coregulators provides the potential for activity. For actual conversion to active functional molecules, however, the coregulators must be regulated by a variety of post-translational modifications, such as phosphorylation, ubiquitylation, acetylation, SUMOylation, methylation, etc. In general, coactivators are activated by phosphorylations and mono- ubiquitylations. Protein–protein interactions in the large coactivator complexes are regulated by acetylations and methylations. Coactivators are inactivated by SUMOylation and degraded after poly- ubiquitylations. These general rules often vary for a given coactivator. Considering the crucial role that post-translational modifications play in coactivator function, it is logical to assume that the roles of signaling pathways that contain these modifying enzymes also play important roles. Since the signaling pathways have certain cell specificities and are subject to environmental stimuli for their regulation, cell context can play a role in selective activities of SRMs.

Because equilibrium reactions are the basis for biology, the promotional and contradictory influences inherent to the cell can affect coactivator function and transcriptional potency. As discussed above, coregulator concentrations are subject to turnover by ubiquitin-dependent and ubiquitin-independent proteasomal degradation pathways, whose activities can be abrogated by certain counteracting kinases. Therefore the cell concentrations and activation of degradation pathways for coregulators can play a role in SRM actions. In addition, in vivo systemic metabolism and selective cellular uptake or metabolism of ligands can sometimes modify SRM activities.

The cell levels of activated coregulators are the primary determinant of tissue-specific SRM activity. Having described herein the complete interacting equations and complexities of coregulator function, it remains that (1) the cellular complement of coregulators and (2) the cell and signaling context are the primary determinants of coregulator function. Consequently, they are the primary determinants of SRM functions.

SRMs are generally mixed antagonist/agonist ligands for receptors. When a receptor is occupied by a mixed antagonist/agonist ligand, the conformation generated in the receptor is neither purely antagonistic nor purely agonistic for activity. Rather, the conformation is intermediate for both functions (Fig 3). A pure agonist induces a receptor conformation that has a strong affinity for coactivators. A pure antagonist induces a receptor conformation that has a strong affinity for corepressors. The mixed antagonist/agonist ligand induces an intermediate conformation that, in turn, is intermediate in its affinity for both coactivators and corepressors. In other words, this receptor conformation is programmed by the local concentrations of activated coactivators and corepressors. The mechanism will obey the laws of physical chemistry. If the cellular concentration of preferred...
coactivators is high (or corepressors low), then the receptor is forced into the active conformation by the excess of coactivators and receptor-dependent gene expression takes place. If the cellular concentration of preferred corepressors is high (or coactivators low), then the receptor is forced into the inactive conformation by the excess of corepressors and receptor-dependent gene expression is shut down. Since activation of coregulators occurs by post-translational modifications, the status of the cell signaling pathways that produces these post-translational modifications is an overarching modulator of SERM activity.

With this background of the physiologic basis for SERM action, it is now appropriate to meld these emerging data with the current applications of SERMs in the clinic and the evolving ideas about drug resistance to SERMs.

The clinical application5 of the laboratory strategy of long-term antihormonal therapy47–49 as an adjuvant to treat breast cancer has now become the standard of care. Two approaches to antihormonal therapy have occurred during the last three decades: long-term treatment to block estrogen-stimulated growth at the level of the tumor ER39 and, subsequently, the use of aromatase inhibitors to block estrogen biosynthesis in postmenopausal patients.5 It is clear that the aromatase inhibitors offer advantages over tamoxifen as adjuvant treatments for postmenopausal patients; there are fewer adverse effects (blood clots and endometrial cancer), and aromatase inhibitors have a small but significant improved efficacy.40,41 However, substantial numbers of postmenopausal patients continue to receive tamoxifen treatment for postmenopausal patients; there are fewer adverse effects (blood clots and endometrial cancer), and aromatase inhibitors have a small but significant improved efficacy.40,41 However, substantial numbers of postmenopausal patients continue to receive tamoxifen treatment either for economic reasons or because they are hysterectomized and at low risk for blood clots (low body mass index or they are athletically active). Postmenopausal women who have completed 2 to 5 years of adjuvant tamoxifen are also eligible for a further 5 years of antihormonal therapy with an aromatase inhibitor.42–44 However, the veteran SERM tamoxifen is still the antihormonal treatment of choice for premenopausal patients and the antihormonal treatment for ductal carcinoma in situ (DCIS),53 and remains the appropriate treatment to reduce breast cancer risk in premenopausal women at elevated risk.56 It is important to stress that premenopausal women treated with tamoxifen do not experience elevations in endometrial cancer and blood clots, so the risk/benefit ratio is strongly in favor of tamoxifen treatment.57

The development of raloxifene48 has created a new therapeutic dimension. Raloxifene is used either as a treatment and preventive for osteoporosis but with a quantifiable decrease in the incidence of breast cancer,49,50 or as an agent for the reduction of breast cancer incidence in high-risk postmenopausal women.51 The advantage of raloxifene as a SERM is that there are no increases in endometrial cancer51,52 incidence previously noted with tamoxifen in postmenopausal women.53

The target site-specific actions of tamoxifen and raloxifene in breast and endometrial cancer were first noted in the laboratory,54,55 but the question to be asked is why. On the basis of our earlier arguments about the mechanism of actions of SERMs, studies of the cellular context and coactivator content demonstrate the tissue-specific actions of tamoxifen and raloxifene in the uterine cancer cell.56

Overall, the SERM concept10,11 clearly works in clinical practice, but the use of long-term SERM treatment regimens raises the important issue of the eventual development of drug resistance. Laboratory studies have already shown that long-term SERM treatment changes the pharmacology from an antiestrogen- to SERM-stimulated growth.37,58 This acquired resistance is a topic of immediate clinical concern.

There are currently three possible mechanisms for drug resistance to tamoxifen. Either the patient can influence the effectiveness of tamoxifen via alterations in metabolism, or the ER-positive tumor is or can become refractory to treatment. These mechanisms are illustrated in Figure 4.

**Metabolic Resistance**

The metabolic activation of tamoxifen occurs via demethylation to N-desmethyltamoxifen and subsequently transformation to the hydroxy metabolite endoxifen.59–61 This topic has recently been reviewed61 and will therefore be mentioned only briefly. Metabolic activation appears to be important for tamoxifen to acquire potent antiestrogenic and antitumor activity. Although large-scale prospective clinical trials have not been completed to prove the hypothesis definitively in large populations, there is sufficient preliminary data to warrant further study. Extensive laboratory studies demonstrate62 that endoxifen is formed by the CYP2D6 enzyme system. However, there are wide variations in the CYP2D6 enzyme in the population that can influence drug metabolism. The wild-type CYP2D6 enzyme is referred to as CYP2D6*1, whereas CYP2D6*4/*4 is a null variant. It is estimated that approximately 10% of the population have CYP2D6 variants, so the case can be made that these patients should be considered for other antiestrogenic interventions (eg, aromatase inhibitors). Another dimension for consideration is the control of menopausal symptoms, especially hot flushes. If tamoxifen is a prodrug and needs to be converted to endoxifen to achieve maximal antitumor activity at the tumor ER, then these same patients may have severe hot flushes. The selective serotonin reuptake inhibitors (SSRIs) have been found to be of value to treat hot flashes. The widespread use of tamoxifen as a long-term adjuvant therapy, especially in premenopausal patients, has naturally increased SSRI use. Unfortunately, the SSRIs such as fluoxetine and paroxetine are potent inhibitors of the CYP2D6 enzyme.63 Therefore, symptom treatment has the potential to undermine the efficacy of tamoxifen if the incorrect SSRI is employed. Venlafaxine has a very low affinity for the CYP2D6 enzyme system and may be the agent of choice for treatment of hot flushes.63 It should, however, be pointed out that there is no substantial clinical evidence to support this conclusion. A larger body of prospective clinical data is required to confirm the admittedly compelling preliminary studies.

**Intrinsic Resistance**

A proportion of ER-positive tumors are intrinsically resistant to tamoxifen therapy. Historically, metastatic breast cancer that is ER and PR positive is approximately 80% responsive to antihormonal therapy (endocrine ablation or tamoxifen) whereas tumors that are ER positive but PR negative are only 40% responsive to antihormonal therapy.64,65 We have known for about 20 years that enhanced growth factor signaling via the human epidermal growth factor receptor 1 (HER-1; EGFR) pathway impairs estrogen induction PR in breast cancer cells66 and enhanced paracrine growth factor stimulation undermines that effectiveness of antiestrogen treatment at the ER.67,68
These earlier observations have recently been confirmed and extended using breast cancer cells artificially transfected with insulin-like growth factor receptor and using large tumor databases. Tumor cell drug resistance to tamoxifen develops very quickly (8 weeks) in athymic mice with HER-2/neu engineered MCF-7 cells compared with the natural process of more than 6 months. Tamoxifen acts as an agonist in experimentally engineered breast cancer cells with high levels of the HER-2/neu growth factor receptor and the coactivator SRC3 (AIB1).

In another approach, the possible connection between HER-2/neu, ER, PR and tamoxifen resistance has been evaluated in a tissue database linked to clinical outcomes. Intrinsic tamoxifen resistance is associated with HER-2/neu–, ER–, PR– tumors that have an increase in coactivator SRC3 (AIB1) levels. Although the actual number is a small group of approximately 10% to 15% breast cancer patients, it does perhaps provide a clue to test who should avoid tamoxifen treatment.

The idea that growth factor receptor could be a predictor of SERM resistance has recently been extrapolated to explain the reason for aromatase inhibitors being superior to tamoxifen as adjuvant therapy. A retrospective analysis shows that patients with ER-positive, PR-negative tumors are more likely to respond to aromatase inhibitors than to tamoxifen. However, the conclusions, though attractive, require confirmation with prospective studies because of inconsistencies with the results from other direct trial databases comparing tamoxifen with an aromatase inhibitor and the recent reevaluation of the steroid receptor database in the original study of tamoxifen and anastrozole.

Acquired Resistance

Laboratory studies show that the treatment of athymic mice implanted with ER-positive, PR-positive MCF-7 tumors with continuous tamoxifen will eventually develop tamoxifen-stimulated tumors that will grow in response to either tamoxifen or estradiol. Either no treatment or treatment with the pure antiestrogen fulvestrant results in no tumor growth. Because no treatment in the ovariectomized athymic mouse is equivalent to treatment with an aromatase inhibitor and fulvestrant destroys the ER, one could conclude that tumor growth is prevented in the absence of a stimulatory signal transduction pathway. This hypothesis is consistent with the clinical observation that anastrozole and fulvestrant treatment are equivalent after the failure of tamoxifen therapy.

Goss et al demonstrated that patients with ER-positive tumors and treated for 5 years with tamoxifen continue to be responsive to subsequent treatment with 5 years of the aromatase inhibitor letrozole. This result could be interpreted as the slow development of acquired resistance by the breast cancer micrometastases during 5 years of tamoxifen so that these patients respond to a non–cross-resistant therapy that prevents tumor growth by blocking the ability of the patient to synthesize estrogen. Thus, the use of letrozole after tamoxifen is incrementally building on the already established long-term antitumor effect of tamoxifen that lasts for at least 10 years after the cessation of adjuvant therapy.

Laboratory models of drug resistance should replicate the duration of SERM administration to patients. Most laboratory models of antihormone resistance are either engineered with stable transfection of the HER-2/neu gene into MCF-7 cells or reflect the early development of resistance (SERM-stimulated growth) to treatment. This later form of resistance is consistent with tamoxifen failure during the

CONSEQUENCES OF LONG-TERM ANTIHORMONE THERAPY

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treatment of metastatic disease. Under these clinical circumstances, tamoxifen treatment is effective for approximately 1 year. This form of SERM resistance is referred to as phase I. However, tamoxifen is used as an adjuvant therapy for 5 years, and it is reasonable to suggest that raloxifene will need to be administered for 10 years or more to maintain effectiveness as an antiosteoporosis medicine. Current studies show that up to 8 years of raloxifene reduces the majority of (65%) but not all ER-positive breast cancers. Some tumors must, therefore, be become raloxifene resistant.

The repeated transplantation of MCF-7 breast tumors into successive generations of tamoxifen-treated ovariectomized athymic mice for more than 5 years replicates the exposure of tumor cells to adjuvant tamoxifen. This approach to study SERM resistance results in a continuing dependence on tamoxifen to produce growth, but cross-resistance with the SERMs toremifene and raloxifene develops and a significant change in the response of tamoxifen or raloxifene resistant cells to physiologic estradiol. The signaling pathways for estrogen no longer support growth, but initiate apoptosis by inducing fas receptor, rapidly reducing levels of HER-2/neu and reducing nuclear factor κ B (NFκB) levels. This form of SERM resistance is referred to as phase II resistance. As might be expected, the pure antiestrogen fulvestrant can completely prevent tumor growth in animals. Paradoxically, when combined with physiological estrogen, fulvestrant not only reverses the apoptotic actions of estrogen but also causes robust tumor growth. The mechanism for this therapeutically relevant observation is unclear, but may involve a dramatic upregulation of HER-2 and HER-3 but may also involve the recently described ligand (estrogen, SERM, fulvestrant) activator G protein GPR30. It is possible that this novel observation may have value to plan an appropriate strategy to use fulvestrant plus an aromatase inhibitor as a third-line endocrine therapy. The widespread clinical use of aromatase inhibitors now brings up the question of the consequences of the long-term use of aromatase inhibitors as adjuvant therapies. There will be an eventual development of drug resistance.

Early studies of estrogen deprivation in cell culture demonstrated that cellular ER levels and spontaneous cell replication increase. Subsequent studies demonstrated that the cells initially become supersensitized to the growth properties of minute quantities of estrogen, but as the duration of estrogen deprivation is extended, the cells respond to estrogen with the initiation of apoptosis. This observation has been used to explain the earlier application of high-dose estrogen therapy to treat postmenopausal women with metastatic breast cancer. However, estrogen-deprived cell lines only need very low concentrations of estrogen in the postmenopausal range (inM) to initiate apoptosis. Cell death occurs through an increase in pro-apoptotic genes and can be enhanced by specifically reducing the synthesis of bcl-2. These preclinical studies are being translated to clinical trials by destroying phase II antihormone-resistant breast cancer cells with limited low-dose estrogen therapy followed by maintenance with further treatment with an aromatase inhibitor treatment.

An alternate approach to study the development of drug resistance to aromatase inhibitors in vivo utilizes ER-positive MCF-7 breast cancer cells stably transfected with the CYP19 aromatase enzyme gene. The cells grow into tumors in athymic mice treated with the enzyme substrate androstenedione that is converted to estrone. The model has been used effectively to examine the integration of SERM and aromatase inhibitor therapy and has effectively replicated the clinical experience. Results not only clearly demonstrate the efficacy of aromatase inhibitors when compared with tamoxifen but also demonstrate the development of resistance to aromatase inhibitors. Aromatase resistant tumors become more dependent on growth factor receptor pathways via mitogen-activated protein kinase.

Overall, the basic knowledge of SERM action and the development of laboratory models of antihormonal resistance are proving invaluable to identify molecular targets for future advances in cancer therapeutics. Important clues about the pivotal role of SRCs in SERM drug resistance and tumor cell survival are already apparent. We predict that further progress in cancer cell biology will occur through an enhanced investment to understand the modulatory mechanisms of NRs and their coactivator partners. The new knowledge will create unanticipated opportunities to control cancer in the future.

**FUTURE POTENTIAL FOR NEW SRM DEVELOPMENT**

With the advent of this recent knowledge of the molecular mechanisms of action of transcriptional regulators such as NRs and coregulators, new insights to drug development are rapidly becoming available. The discovery of tamoxifen as a SERM and the successful development of additional SERMs such as raloxifene, have encouraged exploitation of the SERM concept by pharmaceutical companies to discover additional new SRM ligands for other NRs. Some examples are selective progestin modulators (SPRMs) that inhibit uterine cancer but are devoid of stimulatory action in the breast; selective androgen receptor modulators (SARMs) that are anabolic for muscle and bone, but spare the prostate; selective glucocorticoid receptor modulators (SGRMs) that are strongly anti-inflammatory but do not induce glucose intolerance and connective tissue destruction; and selective peroxisome proliferator-activated receptor γ (PPARγ) receptor modulators (SPARMs) that promote insulin sensitivity. All of the foregoing examples are under current development or are being tested in clinical trials. In the case of each of these SRMs, the molecular mechanisms and pathways for their efficacy described herein represent the guiding principles for their tissue-specific actions and represent a substantial health care return for the investment in basic mechanistic scientific research.

**AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

The author(s) indicated no potential conflicts of interest.

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