A Century of Deciphering the Control Mechanisms of Sex Steroid Action in Breast and Prostate Cancer: The Origins of Targeted Therapy and Chemoprevention

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Abstract

The origins of the story to decipher the mechanisms that control the growth of sex hormone–dependent cancers started more than 100 years ago. Clinical observations of the apparently random responsiveness of breast cancer to endocrine ablation (hormonal withdrawal) provoked scientific inquiries in the laboratory that resulted in the development of effective strategies for targeting therapy to the estrogen receptor (ER; or androgen receptor in the case of prostate cancer), the development of antihormonal treatments that dramatically enhanced patient survival, and the first successful testing of agents to reduce the risk of developing any cancer. Most importantly, elucidating the receptor-mediated mechanisms of sex steroid–dependent growth and the clinical success of antihormones has had broad implication in medicinal chemistry with the synthesis of new selective hormone receptor modulators for numerous clinical applications. Indeed, the successful translational research on the ER was the catalyst for the current strategy for developing targeted therapies to the tumor and the start of “individualized medicine.” During the past 50 years, ideas about the value of antihormones translated effectively from the laboratory to clinical settings; who would one treat? The mechanism was also unknown until Allen and Doisy (6), using an ovariec-tomized mouse vaginal cornification assay, showed that a principle, that they called estrogen (identified as estrone, the principal steroid), was present in ovarian follicular fluid. Their major advance set the scene for the subsequent breakthroughs in molecular endocrinology and therapeutics in the latter half of the 20th century (Fig. 1).

Beginnings at the Dawn of the 20th Century

Schinzinger (1) is credited with suggesting that oophorectomy could be used to treat breast cancer; however, this suggestion did not seem to have been adopted. In contrast, the report by Beaston (2) that oophorectomy could initiate a regression of metastatic breast cancer in two premenopausal women was a landmark achievement. Although it is often stated that Beaston’s work was empirical clinical research, the rationale to conduct an oophorectomy was, in fact, an example of early translational research. Beaston was aware of the essential role of removing the ovary in maximizing milk production in cows. He reasoned there was potentially some factor that traveled in the blood supply to the breast as there was no known connection through the nerves. Interestingly enough, he also conducted laboratory experiments in rabbits before his clinical experiment, so the work was bench-to-bedside (2). By 1900, Stanley Boyd (3) had assembled the results of all the available clinical cases of oophorectomy to treat breast cancer in Great Britain in perhaps the first “clinical trial.” Boyd concluded that only one-third of metastatic breast tumors responded to oophorectomy. This clinical result and overall response rate has remained the same to this day.

Unfortunately, responses were of limited duration and enthusiasm waned that this approach was the answer to cancer treatment. The approach of endocrine ablation was only relevant to breast cancer (and subsequently prostate cancer; ref. 4), thus, the approach was only effective in a small subset of all cancer types. At the dawn of the 20th Century, there was no understanding of the endocrine system or hormones. Nevertheless, laboratory studies started to decipher the biological control mechanisms responsible for the clinical observations.

Links between Sex Steroids and Cancer

The trend in breast cancer research in the early years of the 20th century was to use inbred strains of mice to study the growth and incidence of spontaneous mammary cancer. Lathrop and Loeb (5) found that before age 3 months was the optimal time for oophorectomy to prevent the development of mammary cancer, but obviously, this knowledge could not be translated to the clinical setting; who would one treat? The mechanism was also unknown until Allen and Doisy (6), using an ovariec-tomized mouse vaginal cornification assay, showed that a principle, that they called estrogen (identified as estrone, the principal steroid), was present in ovarian follicular fluid. Their major advance set the scene for the subsequent breakthroughs in molecular endocrinology and therapeutics in the latter half of the 20th century (Fig. 1).

The idea that breast cancer might be a preventable disease was extended by Professor Antoine Lacassagne (7, 8) who first showed that estrogen could induce mammary tumors in mice. Lacassagne (9) hypothesized, “If one accepts the consideration of adenocarcinoma of the breast as the consequence of a special hereditary sensibility to the proliferative action of oestrone, one is led to imagine a therapeutic preventive for subjects predisposed by their heredity to this cancer. It would consist—perhaps in the very near future when the knowledge and use of hormones will be better understood—in the suitable use of a hormone, antagonistic or excretory, to prevent the stagnation of oestrone in the ducts of the breasts.” However, when Lacassagne stated his vision at the annual meeting of the American Association for Cancer Research in Boston in 1936, there were no lead compounds that antagonized estrogen action, but the Allen Doisy mouse assay could be used to study structure activity relationships to find synthetic estrogens. Within a decade, a landmark discovery was to occur in “chemical therapy” that was to expand the treatment of metastatic breast cancer to include postmenopausal women who are, in fact, the majority who develop metastatic disease.
During the 1930s, there were significant advances in the knowledge of the precise structural requirements for estrogen action in its target tissue, the vagina. Synthetic compounds based on stilbene (10, 11) and triphenylethylene (12) were screened using the Allen Doisy ovariectomized mouse vaginal cornification assay to define compounds with optimal structures and duration of estrogen action. Sir Alexander Haddow found that carcinogenic polycyclic hydrocarbons would cause tumor regression in animals. However, these could not be used to treat humans. The nonsteroidal triphenylethylene-based estrogens had similar structures to polycyclic hydrocarbons and also caused tumor regression in animals. With this clue, Sir Alexander Haddow (13) used the first chemical therapy to treat patients. His results published in 1944 showed that high-dose estrogen therapy was effective in causing tumor regressions in postmenopausal patients with breast cancer and men with prostate cancer. There was, however, no understanding of a mechanism. Indeed he stated in 1970: "In spite of the extremely limited practicability of such measure [high dose estrogen], the extraordinary extent of tumor regression observed in perhaps 1% of postmenopausal cases has always been regarded as of major theoretical importance, and it is a matter for some disappointment that so much of the underlying mechanisms continues to elude us" (14). These experimental data were also an apparent paradox as endocrine ablation to remove estrogens and their precursors was the dogma of the time (15).

In the past 50 years, the progress in deciphering the control mechanisms of estrogen action in breast cancer (and androgen action in prostate cancer), has accelerated with advances in technology and an understanding of cell biology. But progress in research does not travel in straight lines, yet chance observations can create a major breakthrough. This has happened repeatedly in the story of the treatment and prevention of breast cancer.

Conceptual Progress through Scientific Serendipity

It is perhaps relevant to illustrate a few astute observations by scientists that accelerated progress immensely in deciphering the complexities of hormone action and the control of breast cancer growth.

Sir Charles Dodds (11) is credited with the synthesis of the potent synthetic estrogen diethylstilbestrol (Fig. 2) that was subsequently used for the treatment of both prostate cancer and breast cancer, and regrettably was also applied to prevent recurrent abortions (16), which caused an increase in clear cell carcinoma of the vagina in the children (17). During the race to describe the minimal molecular structure that would trigger vaginal cornification in the ovariectomized mouse vagina, controversy erupted in the 1930s over the reproducibility of results concerning the compound anethole. The authors were minimalistic in reporting the synthetic methodology, so replication proved impossible to create the correct biology. Rather the product was correct, but the method used by the original authors was not reported accurately and caused dimerization of anethole to an impurity dianethole an estrogen. This active impurity was structurally similar to parallel research endeavors that concluded with the synthesis of the potent estrogen diethylstilbestrol. Thus, the purity of chemicals for testing was critical for successful science.

A similar story was also immensely important in allowing scientists to understand the direct actions of estrogen on the breast cancer cell in vitro. The MCF-7 estrogen receptor (ER)-positive breast cancer cell line (18) has been the work horse for the study of estrogen-stimulated growth. However, early examination of MCF-7 cells in the 1970s could not uniformly show estrogen-stimulated growth. Antiestrogens inhibited the apparently constitutive growth of MCF-7 cells, but estradiol did reverse the inhibitory actions of...
antiestrogens on growth (19). The mystery deepened when studies in vitro could not show estrogen-stimulated growth but MCF-7 cells inoculated into athymic mice would grow into tumors only with estrogen treatment. There was clearly a second factor required for estrogen-stimulated tumor growth in vivo! (20).

The astute observations of John and Benita Katzenellenbogen solved the mystery of why estrogen did not stimulate MCF-7 breast cancer cell growth in vitro. It seems that all cells had been grown for more than a decade in standard medium containing large concentrations of a pH indicator called phenol red. The Katzenellenbogens realized that the structure of phenol red was similar to nonsteroidal estrogens and removal of the indicator from cell culture media caused cell growth rate to decrease and only then would exogenous estrogen cause growth (21). In other words, the cells were already growing maximally in phenol red containing medium. Subsequent studies revealed that the culprit was, in fact,
a partially dimerized chemical contaminant of phenol red. This critical technical advance permitted all of the subsequent understanding of the molecular biology of direct estrogen action.

Leonard Lerner (22) was a young research endocrinologist employed by Merrell Dow to study nonsteroidal estrogen pharmacology. He noticed that the structure of one of the compounds being tested for the control of coronary artery disease was a triphenylethanol similar to the estrogenic triphenylethylenes and he asked to test this chemical as an estrogen. To his surprise, the compound, subsequently renamed MER25 or ethamoxystriphetol, was antiestrogenic in all species tested and had no estrogen-like actions in any animal tests. Lerner (22) had discovered the first nonsteroidal antiestrogen. Although the compound was too toxic and not potent enough for clinical use, Lerner went on to be involved in the discovery of the first triphenylethylene antiestrogen called chloramiphene (MRL41) later to be known as clomiphene (23). Originally, the nonsteroidal antiestrogens were predicted, based on animal studies, to be potent postcoital contraceptives, which in the early 1960s had a huge potential market as “morning after pills.” However, clomiphene did exactly the opposite; it induced ovulation in women (23). Enthusiasm waned and there was general disinterest in this area of research until ICI 46,474, another nonsteroidal antiestrogen discovered in the fertility program of ICI Pharmaceutical Ltd (now AstraZeneca; ref. 24) was reinvented as the first targeted therapy for breast cancer and the first chemopreventive for any cancer (25).

A Target for Treatment and Prevention

The early theory for estrogen action in its target tissues, e.g., uterus, vagina, etc., was that there was chemical transformation between estrone and the less abundant 17β estradiol (Fig. 2) to control the redox potential of the tissue environment. In the late 1950s, Jensen (Fig. 3) and Jacobsen (26) chose another approach at the Ben May Laboratories of the University of Chicago. They synthesized (6, 7) [3H] estradiol (Fig. 2) with very high-specific activity. After its injection into the immature female rats, the unchanged steroid bound to and was retained by the estrogen target tissues: the uterus, vagina, and pituitary gland. In contrast, [3H] estradiol bound to, but was not retained, by nontarget tissues, e.g., muscle, lung, heart. There was clearly a receptor mechanism at play that could be blocked (27) by the coadministration of the first nonsteroidal antiestrogen MER-25 (22).

The mystery of why only about one third of advanced breast cancers responded to either endocrine ablation (3) or high-dose estrogen therapy (15) was solved by the application of basic endocrinology to the practical issue of excluding women with metastatic breast cancer who would not significantly benefit from

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**Figure 3.** Professor Charles Huggins (left) and Elwood Jensen were to receive the Nobel Prize for Physiology and Medicine (1966) and the Lasker Award (2004) for their work on androgen action in cancer and the role of ER in physiology and cancer, respectively.
unnecessary endocrine ablative surgery (oophorectomy, adrenalectomy, or hypophysectomy). The ER was found to be an extractable protein from the rat uterus that would bind [3H] estradiol in the extraction cytosol (28, 29). During the late 1960s, numerous methods were described to identify and quantitative ER levels in tumor biopsies (30) and these data were subsequently correlated with clinical outcomes in metastatic breast cancer (30). Breast tumors without the ER were unlikely to respond to endocrine ablation and therefore should not be treated with this modality. The ER assay was introduced as the standard of care in the mid-1970s to predict endocrine responsiveness to endocrine ablation. It should be stressed that tamoxifen was not available in medical practice until the Food and Drug Administration (FDA) approved this “hormone therapy” in December 1977 for the treatment of metastatic breast cancer in postmenopausal women (23). Indeed, research with the value of the ER assay to predict responsiveness to antiestrogens was unconvincing (23) and the value of adding another hormone therapy to the treatment armamentarium was uncertain. In the 1970s, all hopes in medical oncology were focused on discovering the correct combination of high dose cytotoxic therapies to cure breast cancer much in the same way as both childhood leukemias and Hodgkin’s Disease had been cured. This was not to be but translational research took another route; using the ER as a drug target instead of as a predictive test for endocrine ablation (31).

An Unlikely Therapeutic Solution

Professor Paul Ehrlich (1854–1915) established a model for the development of chemical therapies (chemotherapy) to treat infectious disease. A range of chemical therapies would be synthesized to study structure function relationships in appropriate laboratory models that replicated human disease (32). A clinical study would then be performed on the most promising candidate. Ehrlich’s pioneering work to develop Salvarsan for the successful treatment of syphilis is a landmark achievement (32). He was, however, unsuccessful in applying the same principles to cancer chemotherapy. Indeed, even as recently as 1970, Sir Alexander Haddow (14) stated that there was unlikely to be a “chemotherapia specifica” like Paul Ehrlich envisioned because cancer was so similar to the tissue of origin. There was also no target or effective tests or models to predict efficacy in cancer treatment before administration to the patient. The key to the successful development of tamoxifen, a failed contraceptive (23), was the application of Ehrlich’s principles of developing an effective treatment strategy by using disease specific laboratory models and the use of the tumor ER as a target for drug action (25).

Available laboratory models for the study of the antitumor actions of antiestrogenic drugs were strains of mice with a high incidence of spontaneous mammary tumors (5) or the carcinogen-induced rat mammary carcinoma (33). The mouse models had fallen out of fashion with the discovery of the “Bittner milk factor,” a virus that transmits mammary carcinogenesis to subsequent generations through the mother’s milk (34). The research community also began to realize that breast cancer was not a viral disease. Nevertheless, the knowledge of mouse mammary carcinogenesis proved to be pivotal for developing precise and targeted promoters to initiate mammary cancer with oncogenes using transgenic mice (35). Another problem with tumor testing of tamoxifen in mice was the unusual observation that tamoxifen, orICI 46,474 as it was then known, was an estrogen in the mouse (24, 36). This pharmacologic peculiarity became important later with the recognition of selective ER modulation (37). Most importantly, work did not advance quickly in the 1960s and early 1970s, as there was no enthusiasm about introducing a new “hormonal therapy” into clinical practice (25). All early compounds had failed to advance past early clinical studies and only tamoxifen was marketed (23) for the induction of ovulation or the general treatment of late-stage breast cancer in postmenopausal women (38–40).

In the late 1960s, the 7,12-dimethylbenz(a)anthracene–induced (DMBA) rat mammary carcinoma model (33) was extremely fashionable for research on the endocrinology of rat mammary carcinogenesis (41, 42). However, the parallels with breast cancer are few, as the tumors do not metastasize and are regulated primarily by prolactin secreted by the pituitary gland in direct response to estrogen action (43). Be that as it may, there was no alternative. Therefore, the DMBA rat mammary carcinoma model was adapted to determine the appropriate strategy for the use of antihormonal therapy as an adjuvant. At that time in the mid-1970s, the early adjuvant trials with tamoxifen did not target patients with ER-positive breast cancer and used only short-term (1 year) tamoxifen treatment to avoid premature drug resistance. This duration of tamoxifen that was selected as the antiestrogen only controlled the growth of metastatic breast cancer for about a year (39). The value of short- and long-term (1- or 6-month treatment equivalent to 1 or 6 years of adjuvant treatment in patients) antihormone administration was determined starting treatment 1 month after DMBA administration to 60-day-old Sprague-Dawley rats. Long-term therapy was remarkably effective at controlling the appearance of mammary tumors and was far superior to short term treatment (44, 45). The concepts of targeting the ER and using long-term adjuvant therapy effectively translated through clinical trials to improve national survival rates for breast cancer (46, 47).

Targeting Treatment for Breast Cancer

The early clinical work of Santen (48) established the practical feasibility of using aminoglutethimide, an agent that blocks both adrenal steroidogenesis and the CYP19 aromatase enzyme to stop conversion of testosterone and androstenedione to estradiol and estrone, respectively. Unfortunately, aminoglutethimide must be given with a natural glucocorticoid; therefore, long-term therapy is not a practical possibility. Brodie and coworkers (49, 50) advanced knowledge of the specific targeting of the CYP19 aromatase enzyme with the identification and subsequent development of 4 hydroxyandrostenedione (51) as the first practical suicide inhibitor of the aromatase enzyme (Fig. 4). Incidentally, the pivotal work with both tamoxifen and 4-hydroxyandrostenedione (Figs. 2 and 4) was initiated at the Worcester Foundation for Experimental Biology in Massachusetts in the early 1970s (52). Brodie’s contribution eventually became the catalyst to create a whole range of agents (e.g., anastrozole; Fig. 3) targeted to the aromatase enzyme for the treatment of breast cancer in postmenopausal women (53). The clinical application of aromatase inhibitors has reduced the side effects noted with tamoxifen in postmenopausal women such as blood clots and endometrial cancer and there has been a small but significant improvement in disease control for the postmenopausal patient when results are compared with tamoxifen (54, 55).

However, recent research into the pharmacogenetics of tamoxifen has suggested that CYP2D6 enzyme product is important for
metabolism to the active antiestrogen endoxifen (4-hydroxy-N-desmethyltamoxifen; ref. 56), and the use of certain selective serotonin reuptake inhibitors to reduce hot flashes seems to be contraindicated because of drug interaction at the CYP2D6 enzyme (57, 58). Current research is also exploring the hypothesis that a mutated and ineffective CYP2D6 gene product undermines the therapeutic activity of tamoxifen (57, 58). It may be that patients could eventually be selected for optimal effective tamoxifen treatment in cases of ER-positive breast cancer. This would be worthwhile for the chemoprevention of breast cancer. Clearly, the identification of patients for optimal long-term use of tamoxifen should exclude those high-risk women with a mutant CYP2D6 gene who choose to use chemoprevention, as tamoxifen treatment may possibly be suboptimal.

Chemoprevention of Breast Cancer

In the middle of the 1970s, Sporn (59) advanced the concept of the chemoprevention of cancer and strongly advocated this approach as the optimal and clearly most rational way to reduce the burden of cancer. Practical chemoprevention articulated by Lacassagne (9) has its foundations with the finding that tamoxifen prevents DMBA-induced rat mammary carcinogenesis (60, 61). These laboratory findings (45, 60, 61) and the subsequent clinical finding that adjuvant tamoxifen treatment reduces the incidence of contralateral breast cancer (62) prompted Powles (63, 64) to initiate the first exploratory trial to test the worth of tamoxifen to reduce the incidence of breast cancer in high risk women. Although numbers were small, the Powles study did ultimately show the ability of tamoxifen to reduce breast cancer incidence many years after the treatment had stopped (65). In contrast, the large study by Fisher (66, 67) definitively showed the efficacy of tamoxifen to reduce the incidence of ER-positive breast cancer initially and continues to do so after therapy stops in both premenopausal and postmenopausal women at high risk. Tamoxifen became the first medicine approved by the FDA for risk reduction of any cancer. However, concerns based on laboratory findings (68), about the potential of tamoxifen to increase the risk of endometrial cancer in postmenopausal women and the carcinogenic potential of tamoxifen as a hepatocarcinogen (69), demanded that there had to be a better way to reduce the risk of breast cancer as a public health initiative.

The recognition of selective estrogen receptor modulator (SERM) action by nonsteroidal antiestrogens that stimulate some estrogen target tissues but block estrogen-stimulated tumorgrowth in others, (70) introduced a new dimension into therapeutics and advanced chemoprevention. Raloxifene has its origins as a nonsteroidal antiestrogen for the treatment of breast cancer (71, 72) as LY156758 or keoxifene. The drug failed in that indication (73) and further development was abandoned (74). The discovery that both tamoxifen and keoxifene would maintain bone density in ovariectomized rats (75), block rat mammary carcinogenesis (76), but that keoxifene was less estrogen-like than tamoxifen in the rodent uterus (71) and was less effective in stimulating the growth of endometrial cancer, (77) suggested a new therapeutic strategy (78). Simply stated (79): "We have obtained valuable clinical information about this group of drugs that can be applied in other disease states. Research does not travel in straight lines and observations in one field of science often become major discoveries in another. Important clues have been garnered about the effects of tamoxifen on bone and lipids, so it is possible that derivatives could find targeted applications to retard osteoporosis or atherosclerosis. The ubiquitous application of novel compounds to prevent diseases associated with the progressive changes after menopause may, as a side effect, significantly retard the development of breast cancer. The target population would be postmenopausal women in general, thereby avoiding the requirement to select a high-risk group to prevent breast cancer."

Several years later, keoxifene was renamed raloxifene (Fig. 2) and was shown to maintain bone density in osteoporotic or osteopenic women (80), and simultaneously reduce the incidence of invasive
breast cancer without causing an increase in the incidence of endometrial cancer (81). Raloxifene went on to be tested against tamoxifen in the Study of Tamoxifen and Raloxifene trial (82) and was FDA approved both for the treatment and prevention of osteoporosis in postmenopausal women and for the reduction of invasive breast cancer incidence in postmenopausal women at elevated risk. The clinical advances with SERMs-modulating estrogen target tissues has provided exceptional opportunities to treat and prevent multiple diseases. However, for the future it is the study of the molecular events of estrogen action that holds the promise of further breakthroughs in patient care.

Molecular Mechanisms of Estrogen and SERM Action

It is not possible to provide a comprehensive review of the explosion of interest in receptor-mediated molecular mechanisms of action of estrogen, so the reader is referred to significant reviews to appreciate the evolution of the topic (83, 84). What will be presented is an evolving guide to current thinking. There are two ERs called α and β (Figs. 5 and 6). The receptor ERα is the traditional ER (26, 28), but it should be stressed that the development of monoclonal antibodies to ER (85) was the essential step for ERα cloning (86, 87) that provided the clues to discover ERβ (88). The receptor proteins encode on different chromosomes and have homology as members of the steroid receptor superfamily, but there are distinct patterns of distribution and distinct and subtle differences in structure and ligand binding affinity. An additional dimension that may be significant for tissue modulation is the ratio of ERα and ERβ at a target site. A high ERα/ERβ ratio correlates well with very high levels of cellular proliferation, whereas the predominance of functional ERβ over ERα correlates with low levels of proliferation (89, 90). The ratio of ERs in normal and neoplastic breast tissue may be an important factor for the long-term success of chemoprevention with SERMs.

There is, as a result, much interest in synthesizing ER subtype specific ligands.

There are functional differences between ERα and ERβ that can be traced to the differences in the Activating Function 1 (AF-1) domain located in the amino terminus of the ER (Fig. 6). The amino acid homology of AF-1 is poorly conserved (only 20%). In contrast, AF-2 region located at the C terminus of the ligand binding domain, differs only by one amino acid: D545 in ERα and N496 in ERβ. Because the AF-1 and AF-2 regions are critical for the interaction with other coregulatory proteins and gene transcription, the structural differences between AF-1 provides a clue about the potential functional differences between ERα and β. Studies using chimeras of ERα and β by switching the AF-1 regions show that this region contributes to the cell and promoter specific differences in transcriptional activity. In general, SERMs can partially activate engineered genes regulated by an estrogen response element through ERα but not ERβ (91, 92). In contrast, 4-hydroxytamoxifen and raloxifene can stimulate activating protein-1–regulated reporter genes with both ERα and ERβ in a cell-dependent fashion.

The simple model for estrogen action, with either ERα or ERβ controlling estrogen-regulated events, has now evolved into a fascinating mix of protein partners that have the potential to modulate gene transcription (Fig. 5). It is more than a decade since the first steroid receptor coactivator was first described (93). Now dozens of coactivator molecules are known, and also corepressor molecules exist to prevent the gene transcription by unliganded receptors (94).

It is reasonable to ask how does the ligand program the receptor complex to interact with other proteins? X-ray crystallography of the ligand binding domains of the ER liganded with either estrogens or antiestrogens show the potential of ligands to promote coactivator binding or prevent coactivator binding based on the shape of the estrogen or anti-ER complex (95, 96). Evidence has accumulated that the broad spectrum of ligands that bind to

Figure 5. Molecular mechanisms of estrogens, antiestrogens, and SERMs in estrogen target tissues. The nuclear receptor complex (NRC) that results from ligand binding to either ERα or ERβ can interact with either coactivators (CoA) or corepressors (CoR) to initiate estrogenic or antiestrogenic responses, respectively. The activation of transcription at a promoter site of an estrogen responsive gene occurs through the binding of the complex that is cyclically destroyed through the proteasome and then the reassembling of a new complex.
the ER can create a broad range of ER complexes that are either fully estrogenic or antiestrogenic at a particular target site (97). Thus, a mechanistic model of estrogen action and antiestrogen action (Fig. 5) has emerged based on the shape of the ligand that programs the complex to adopt a particular shape that ultimately interacts with coactivators or corepressors in target cells to determine the estrogenic or antiestrogenic response, respectively.

Not surprisingly, the coactivator model of steroid hormone action has now become enhanced into multiple layers of complexity thereby amplifying the molecular mechanisms of modulation (98). The ER complex with its core coactivator (e.g., SRC3) positions itself in the promoter region of an ER responsive gene and attracts associated molecules that engages RNApolIII to start transcription. However, the complex of associated molecules also acetylates or deacetylates histones on DNA, thereby regulating the exposure of DNA to modulate transcription. Additionally, associated molecules are recruited to the receptor complex that are members of a family of enzymes that ubiquitinylate proteins in the complex for destruction. Estrogen action is therefore a dynamic process of complex assembly and destruction at the target gene (99).

The complicated modulation of estrogen action at individual target sites is challenging to comprehend but provides opportunities to develop new targeted treatments for sex steroids.

Current Insights into Sex Steroid Modulation

The accumulated knowledge about modulating the ER complex through coregulators interacting at AF-2 and AF-1 create new opportunities for novel drug discovery. The target site modulation of the ER with SERMs has been expanded to the androgen receptor (AR) with selective AR modulators (SARM; refs. 100, 101). Existing nonsteroidal SARMs are being used to define tissue specific gene expression that will lead to clinically useful selective anabolic therapies without stimulating the prostate (102).

Studies of the molecular pharmacology of selective nuclear receptor modulators are focused on the relationship between the external shape of the ligand receptor complex and coregulator binding at AF-2 (103, 104). Combinatorial phage display can identify external regions of the receptor complex to map SARM action or create peptide antagonists that will block coactivator binding with potential as new therapies for prostate cancer. Indeed, this approach is now being extended to orphan nuclear receptors that do not need a small ligand for gene regulation (105). Progress with defining cofactors to study the biology of estrogen-related receptor α (ERR-α) is an important advance with significance for new targeted therapeutic agents. The recent description of the role of ERR-α in angiogenesis of ER-negative tumors (106) is a potential practical application of this work.
Posttranslational modifications of sex steroid receptors at AF-1 through phosphorylation cascades have their origins from the cell surface growth factor receptors (107, 108). This knowledge has a potential application to understand the molecular biology of antihormone resistance. However, our evolving knowledge of antihormonal drug resistance has important therapeutic consequences.

**Drug Resistance to SERMs**

The acceptance of the concept of long-term antihormonal therapy to target, treat, and prevent breast cancer (25) raised the specter of drug resistance to SERMs and SARMs. However, the early models of SERM resistance did not reflect the majority of clinical experience. The natural laboratory models of antihormone resistance caused stimulation of tumor growth during a year of therapy (109), and therefore, reflected drug resistance in patients with metastatic breast cancer who are only treated successfully for a year. The early laboratory models of drug resistance did not replicate clinical experience with adjuvant therapy for 5 years. Remarkably, drug resistance evolves (Fig. 7) and the survival signaling pathways in tamoxifen-resistant tumors becomes reorganized so that instead of estrogen being a survival signal, physiologic estrogen now inhibits tumor growth (110). This discovery provides an invaluable insight into the evolution of drug resistance to SERMs and prompted the reclassification of the process through phase I (SERM/estrogen stimulated) to phase II (SERM-stimulated/estrogen-inhibited growth; ref. 111).

This model would also explain the earlier observations (13) why high-dose estrogen therapy was only effective as a treatment for breast cancer in women many years after the menopause. Natural estrogen deprivation had occurred. The process is accelerated and enhanced, however, in patients treated long term with SERMs or aromatase inhibitors so that only low doses of estrogen are necessary to cause experimental tumors to regress. The new knowledge of the apoptotic action of estrogen (or androgen—see next section) could potentially lead to the discovery of a precise apoptotic trigger initiated naturally by steroid hormone receptors (111). Discovery of this apoptotic trigger might result in an application that targets critical survival signals with new drugs.

**Parallel Path of the Prostate**

Charles Huggins (Fig. 3; ref. 112) resurrected the use of endocrine ablation for the treatment hormone-dependent breast cancers. His focus, however, was the regulation of the growth of the prostate gland and the application of that knowledge for the treatment of prostate cancer (4). He received the Nobel Prize for Physiology and Medicine in 1966. The process for deciphering the molecular mechanisms of androgen action in its target tissues and prostate cancer has tended to lag behind the pathfinder estrogen. Nevertheless, the basic model for the regulation of nuclear hormone receptor action is consistent but the details of androgen action are distinctly different than estrogen action, which in turn created novel therapeutic opportunities to stop the biosynthesis of each active steroidal agent. The similarities and differences in the molecular actions of estrogen and androgen action are illustrated in Fig. 8. The two significant differences (yet similarities) in the biosynthetic pathways between estrogens and androgens are as follows: (a) the aromatization of the A ring of testosterone to create the high-affinity ER binding ligand 17β estradiol in women. This bioactivation led to the development of aromatase inhibitors to block estrogen synthesis (50); and (b) the reduction of testosterone to the high-affinity AR binding ligand dihydroxytestosterone in men. This knowledge led to the development of the 5α reductase inhibitor finesteride (Fig. 4) that was tested successfully for risk reduction for prostate cancer in men (113). Unfortunately, as yet, finesteride has failed to advance for use as a chemopreventive for prostate cancer because of overstated concerns about the accelerated development of potentially more aggressive prostate cancers in those men who did not have tumorigenesis prevented. In contrast, aromatase...

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**Figure 7.** The evolution of resistance to selective ER modulators (SERMs: tamoxifen or raloxifene) long-term therapy.
inhibitors have advanced to test their worth as chemopreventive agents (53).

A range of antiandrogenic drugs that competitively block the AR are available in clinical practice (114). Drug resistance to antiandrogen therapy parallels antiestrogen drug resistance (115), and following long-term antihormonal therapy with antiandrogens, androgen induces apoptosis in antiandrogen-resistant prostate cancer cells (116). Recent research has identified high local levels of androgen production as a major form of antihormonal drug resistance (117). As a result, a new therapeutic approach is the development of an inhibitor of androgens biosynthesis from cholesterol (Fig. 8) by blocking 17 hydroxylase/17,20 lyase. A promising compound abiraterone acetate (Fig. 4) is currently being evaluated in clinical trials (118). However, there is also a need to coadminister glucocorticoids so long term therapy must be monitored carefully.

**The Successful Evolution of Targeted Antihormonal Therapy in the 20th Century and Beyond**

The identification of the ER and subsequently the AR as the conduit for hormone-mediated development and growth in breast and prostate cancer, respectively, has had a profound effect on the approach to the treatment and prevention of cancers. These hormone-mediating molecules have proved to be the pathfinders for the development of targeted therapies that transformed the approach to cancer treatment away from the nonspecific cytotoxic chemotherapy approach during the 1950s to 1990s. As a result, there is current enthusiasm about the promise of individualized medicine and tumor-specific therapeutics (25, 119).

The effect of antihormonal therapy for breast cancer has been profound with improvements in patient survival, a menu of medicines is now available to suit individual patient needs and there is a decrease in national mortality rates in numerous countries (47). Additionally, there are now two SERMs (tamoxifen and raloxifene) available to reduce the incidence of breast cancer (67, 82). But progress in our understanding and application of SERMs is more than chemoprevention. The SERM concept (70) has spread to develop tissue-selective drugs for all members of the hormone receptor superfamily (25, 120). An enormous interest in developing selective glucocorticoid receptor modulators, selective progesterone receptor modulators, SARMs, and even agents to treat rheumatoid arthritis is an ongoing therapeutic outcome of translational research for the chemoprevention of breast cancer.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.
Hormone-Dependent Cancer

Acknowledgments

Received 8/21/2008; accepted 1/5/2009; published OnlineFirst 02/03/2009.

Grant support: Dr. V.C. Jordan is supported by the Department of Defense Breast Program under award number BC052777 Center of Excellence [Views and opinions of, and endorsements by the author(s) do not reflect those of the US Army or the Department of Defense], FCCC Core grant NIH P30 CA00927, The Avon Foundation, The Genuardi’s Foundation, and the Weg Fund of Fox Chase Cancer Center.

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