Aromatase inhibitors in relation to other forms of endocrine therapy for breast cancer

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Abstract
Most endocrine therapies for breast cancer inhibit tumor growth by depriving the cell of estrogen or by blocking its receptor. However, some drugs, such as tamoxifen, can bind to the estrogen receptor (ER) and have both estrogenic and antiestrogenic effects, depending on the tissue, cell, or promoter context. These mixed properties may be explained by new information on ER function at the molecular level. Whether a synthetic drug acts as an estrogen or antiestrogen on a specific gene may be dictated by the particular ensemble of ER subtype, receptor interacting proteins, other transcription factors, or specific elements within the promoter of estrogen-regulated genes. Alterations in these other factors may also play a role in resistance to hormonal therapies. Aromatase inhibitors, like ovarian ablation, inhibit growth by lowering the estrogen concentration in blood or in the tumor tissue itself. Aromatase inhibitors are effective even in postmenopausal women with low estrogen concentrations - probably because of the ability of the tumor to become hypersensitive to estrogen after prolonged estrogen deprivation. Given that the ER itself is the prime target for endocrine manipulation, the ideal endocrine therapy may be one that reduces or eliminates ER from the tumor cell. Pure steroidal antiestrogens are then of great interest because, not only do they inhibit ER-induced transactivation of estrogen regulated genes, they also induce ER degradation. Additional clinical trials are necessary to identify the optimal endocrine therapy and optimal sequence of available therapies.

Introduction
The female sex steroid hormone, estrogen, is required for the development and progression of human breast cancer. Various endocrine therapies designed to either decrease the estrogen concentration or to block its effects have been developed, dating back to the first ovariectomies performed at the turn of the century; today, they remain major treatment modalities for treatment of breast cancer patients, both in metastatic stages and as adjuvants. The presence of estrogen receptor (ER) in tumors is required for endocrine therapy to be effective, as ER-negative tumors do not have the molecular machinery necessary to be estrogen responsive (Osborne et al. 1980, Early Breast Cancer Trialists’ Collaborative Group 1998).

The general approach to treatment of patients with metastatic ER-positive breast cancer comprises sequential endocrine therapies, reserving chemotherapy until the tumor becomes refractory to hormonal approaches. Thus, if the patient responds to the initial endocrine therapy, a secondary endocrine therapy is often used when the tumor begins to progress later in the course of the disease. Similarly, third- and fourth-line endocrine therapies are often useful in certain patients. The effectiveness of this sequential approach implies, that when breast tumors become resistant to one therapy, they are not necessarily totally estrogen independent and resistant to all endocrine therapies. Resistance, then, is often specific for the particular treatment, and alternative therapies may still be effective. Given that tumors may remain estrogen dependent over time, loss of ER, although it does occur, is not a common cause for resistance to endocrine therapy (Encarnacion et al. 1993a). To understand how endocrine therapy works and why tumors may become resistant to one therapy but not another, an understanding of the molecular mechanisms of ER function is required.

Molecular targets for endocrine treatment
Endocrine therapy of breast cancer is one of the first therapies for any cancer to use a molecular targeting approach to drug design, and it is still the preferred approach for treating ER-positive breast cancer patients, as evidenced by large scale clinical trials. Advances in understanding of the ER molecular targeting machinery have demonstrated that estrogen is required for the maintenance of expression levels of ER, and that induction of ER degradation results in loss of sensitivity to estrogen. Thus, new endocrine therapies that act on other targets may be beneficial in situations where ER down-regulation results in resistance. For example, a recent phase II clinical trial in postmenopausal women with metastatic breast cancer treated with pure steroidal antiestrogens such as exemestane (Aromasin; AstraZeneca, Wilmington, Delaware) demonstrated that these drugs are also effective in patients with tumors that become hypersensitive to estrogen after prolonged estrogen deprivation (Bergsagel et al. 1999). This suggests that tumors may produce hypersensitive receptors in response to prolonged estrogen deprivation, leading to resistance to tamoxifen and other antiestrogens. Other clinical trials, such as the ongoing study in which patients with metastatic breast cancer are treated with both tamoxifen and aromatase inhibitors, are necessary to identify the optimal endocrine therapy and optimal sequence of available therapies.

References
approach. Most treatments are designed either to reduce the ligand (estrogen) for the ER, or to directly block hormone receptor binding. Of course, when ovarian ablation was first performed nearly a century ago, neither estrogen nor its receptor had been identified; now, the molecular and biochemical events regulating estrogen action are much more clearly defined. Furthermore, in addition to ER, other molecular pathways offer promise for future endocrine or biologic treatments. Retinoic acid receptors, the AP-1 nuclear transcription factor family, various polypeptide growth factors and their membrane receptors, proteins regulating programmed cell death, and paracrine factors influencing important events such as angiogenesis, invasion and metastasis are all provocative targets for prevention or treatment. Although experimental therapies targeting some of these pathways are under investigation, only one, an antibody to the HER-2/neu oncogene, has progressed to use in the clinic in routine management of patients. Thus the ER remains the most important target for endocrine therapy and much has been learned in recent years about its function at the molecular level.

**Estrogen receptor function**

The ER is a nuclear protein that functions as a transcription factor to regulate expression of estrogen-responsive genes (Osborne et al. 1996, Osborne 1998). Some of these estrogen-regulated genes mediate growth and development of the mammary gland, and some as yet unidentified genes are important for the effects of estrogen on tumor cell proliferation. After estrogen or another ligand such as tamoxifen or raloxifene binds to the ER, dimerization of the receptor is induced, which then allows binding of the complex to the estrogen-responsive element (ERE), a region in the promoter of estrogen target genes. The binding of the ER dimer to this promoter region then facilitates transcription of that gene, which eventually results in the synthesis of new proteins that alter the cellular phenotype. Synthetic drugs like tamoxifen were first called antiestrogens because they bind ER and competitively block the effects of estrogen on tumor cell proliferation and on the expression of certain genes. Now, however, we know that synthetic drugs like tamoxifen can have a spectrum of effects, depending on the species, tissue, cell, or gene context (Jordan 1998). In some cases, these ‘antiestrogens’ can be estrogenic, stimulating transcription of genes. In other cases, sometimes in the same cell, they have a predominant antiestrogenic activity. This paradox has not been totally explained, but it may be important, not only for our understanding of the mechanism of ER function, but also for an understanding of how tumors develop resistance to endocrine therapies.

It is now clear that the activity resulting from ligand binding to ER is much more complex than was originally believed. Table 1 lists some of the factors influencing the net transcriptional effects induced by ER. These include the ligands bound to the receptor, the type of ER (ERα or ERβ), a variety of proteins that interact directly or indirectly with the receptor, and specific regions in the promoters of target genes (Osborne 1998). The ligands themselves are important because they may induce different conformational changes in the receptor that can alter its function (Jordan 1998). In addition, the human ER is now known to exist as two separate genes, termed ERα and ERβ.

| Table 1 Factors influencing ER transcriptional activation |
|-------------------------------------------|-----------------|-----------------|-----------------|
| **Ligand (estradiol, tamoxifen, raloxifene, other)** |
| **ER subtype (α or β)** |
| **Receptor interacting proteins (co-activators and co-repressors)** |
| **Basal transcription factors** |
| **Promoter elements (ERE, other)** |

Most of the information available on ER structure and function is derived from studies of ERα, which was cloned many years ago. ERβ, in contrast, was cloned just in the past few years, from both rat and human tissues, and minimal information is available concerning its function (Kuiper et al. 1996, Mosselman et al. 1996). The tissue distribution of ERα and ERβ is somewhat different, however (Kuiper et al. 1996, 1997, Mosselman et al. 1996). This suggests that the two receptors may have distinct functions. At the RNA level, some tissues such as the ovary express relatively equal amounts of both receptors. The uterus, pituitary, and testis appear to express more ERα, whereas the prostate and bladder may express more ERβ. Kidney and liver express exclusively ERα, but lung and brain only express ERβ. ERβ is highly homologous to ERα in several of the important functional domains, including the DNA-binding domain and the ligand-binding domain, suggesting that both receptors can bind the EREs on target genes and that they may also bind similar ligands (Mosselman et al. 1996). The transactivation domains are not homologous, suggesting the possibility that they could activate different genes. Estradiol binds with equal affinities to both receptors, but the so-called antiestrogens, 4-hydroxytamoxifen and ICI 164,384, bind to ERβ more tightly than to ERα. Some plant estrogens also bind with greater affinity to ERβ. ERβ is also present in human breast cancer tissue, in which both receptors would be measured by traditional ligand-binding assays (Dotzlaw et al. 1997). Additional studies are needed to determine whether these two receptor forms...
have different physiologic roles in mammary gland growth and development or in breast cancer progression. If they do have different activities, it is possible that the relative levels of ERα and ERβ in a breast tumor could influence the response to hormonal therapy.

Many other nuclear proteins have been identified that interact with the ER dimer (Horwitz et al. 1996, Glass et al. 1997). Some of these receptor-interacting proteins function as co-activators and are associated with acetyl transferase activity, and they appear to amplify transcriptional activation from the ER. Other interacting proteins function as co-repressors, and they are associated with deactylase activity. The acetylase/deactylase activity may be important for changes in DNA conformation that are crucial for gene transcription. ER co-activators include the SRC family, SRC-1 and SRC-2, CBP/P300, TIF2, TRIP1, and AIB1 (also called SRC-3 or RAC-3) (Horwitz et al. 1996, Anzick et al. 1997, Glass et al. 1997). The last of these is amplified, overexpressed, or both, in a large percentage of human breast cancer specimens, suggesting that it may have an important role in breast cancer progression. Among the co-activators, N-CoR and SMRT appear to be important for antiestrogen function (Hörlein et al. 1995, Jackson et al. 1997, Li et al. 1997). They bind to the ER in the absence of estrogen or in the presence of tamoxifen and are crucial for the antiestrogenic activity of tamoxifen. The relative concentrations of co-activators and co-repressors in a cell may also be important. Higher levels of co-activator such as SRC-1 have been shown to enhance the estrogen-agonist activity of tamoxifen, whereas a relative abundance of co-repressors increases its antagonist function (Jackson et al. 1997, Smith et al. 1997). Tamoxifen-stimulated growth, which has been identified as a potential mechanism of acquired resistance to tamoxifen, could perhaps then be explained by alterations in the expression or function of co-activators or co-repressors in a particular tumor. Reduced levels of N-CoR have been reported in tamoxifen-stimulated compared with tamoxifen-inhibited human breast tumors from an in vivo experimental model (Lavinsky et al. 1998). These receptor-interacting proteins could potentially be important tumor markers, much like ER itself, providing additional predictive information on hormonal responsiveness.

Other studies also show that ligand-bound ER can affect transcription of genes that do not contain an ERE in their promoter. For instance, ER can directly interact with other transcription factors such as the AP-1 transcription factor family, to augment or inhibit the expression of genes regulated by these proteins (Webb et al. 1995). Regions of the promoter other than the classical ERE may also be important, and could provide additional specificity at the gene level (Yang et al. 1996).

Thus, ER function is complex and depends on a variety of other factors. The complexity, however, provides a potential explanation for the mixed agonist/antagonist effects of drugs such as tamoxifen and raloxifene. It is plausible that the particular ensemble of ligands, ER subtype, receptor-interacting proteins and other transcription factors, and the promoter sequences of certain genes, act together to determine whether that ligand will have estrogenic or antiestrogenic qualities. Because of the mixed activities of many ER ligands, it has become popular to classify them as selective estrogen receptor modulators (SERMs). At one end of the spectrum are ligands with pure estrogenic activity, and at the other, ligands with pure antiestrogenic activity; in between are ligands with mixed agonist/antagonist properties, depending on the tissue, cell, or gene context. These selective and specific activities have permitted the development of drugs that may benefit patients in a variety of ways other than their antitumor effects.

**Mechanism of action of various endocrine therapies**

Endocrine therapies can be classified into four general types on the basis of their mechanism of action (Table 2).

**Table 2 Mechanisms of action of endocrine therapies**

| 1. Reduce level of estrogen: ovarian ablation, LHRH agonists, aromatase inhibitors. |
| 2. Antagonize ER function by competitive binding: SERMs, pure antiestrogens. |
| 3. Downregulate ER: steroidal antiestrogens. |

Endocrine ablation therapy such as ovariectomy or ovarian suppression by luteinizing hormone-releasing hormone (LHRH) superagonists inhibit tumor growth by decreasing the level of estrogen available to bind to ER. Aromatase inhibitors also decrease the estrogen concentration in postmenopausal women, by blocking peripheral production of estrogen in adipose tissue and in the tumor itself (Ellis 1998). SERMs and other more pure or complete antiestrogens work by antagonizing ER function; these drugs bind to ER and block the binding of estrogen. Steroidal antiestrogens such as ICI 182,780 (Faslodex) not only antagonize ER transcriptional activation, but also markedly downregulate ER concentrations by inducing ER degradation (Dauvois et al. 1992). The mechanism of action of pharmacologic doses of estrogens, androgens, and progestins remains unclear.
Aromatase inhibitors

Aromatase inhibitors inhibit peripheral conversion of adrenal androgens to estrogens, resulting in lower circulating estrogen concentrations and lower concentrations in tumor tissue (Ellis 1998). An enigma is why they have activity in postmenopausal women, in whom estradiol concentrations are already low. It is possible that tumor estrogen concentrations may not always reflect circulating estrogen concentrations, and that the tumor estrogen concentration in some postmenopausal women may be substantial (Ellis 1998). Alternatively, there is growing evidence to suggest that tumors can ‘reset their estrogen thermostat’: tumor proliferation may slow when the estrogen concentration is reduced, but then the tumor adapts to the lower concentrations of estrogen and tumor growth resumes as a result of estradiol hypersensitivity (Masamura et al. 1995); treatment with an aromatase inhibitor then decreases the estrogen concentration further, again resulting in tumor growth suppression. The idea that breast cancer cells can adapt to low levels of estrogen by enhancing their sensitivity to estrogen has been demonstrated in vitro in cultured breast cancer cells deprived of estrogen for long periods of time (Masamura et al. 1995). In addition, in an in vivo athymic nude mouse model of human breast cancer, tumor regression was shown to occur after estrogen deprivation, but tumor growth resumed after 4-6 months. Growth of these tumors progressing after prolonged estrogen deprivation could be inhibited by antiestrogens, which also suggests that their regrowth was mediated via the ER and that the tumors had adapted to the very low estrogen concentrations (Encarnacion et al. 1993b). These data provide the rationale for further reduction of estrogen concentrations by aromatase inhibitors in postmenopausal women. Whether tumors can further adapt to the very low estrogen concentrations observed during aromatase therapy is not clear.

Optimal endocrine therapy

Because most of the various endocrine therapies have similar antitumor activity, the choice of endocrine therapy is often based on drug toxicity and side effects. Theoretically, though, with our new knowledge about ER function in mind, we might be able to speculate as to which endocrine therapy might be optimal over the long term. SERMs are effective because of their antiestrogenic qualities in other tissues. However, resistance mechanisms may also be partly explained by their intrinsic agonist activity resulting in tumor growth stimulation after a period of growth suppression (Osborne et al. 1991). Ablative therapies inhibit tumor growth because they reduce the circulating and tumor concentrations of estrogen, but because the tumor can adapt to low estrogen concentrations, the duration of remission is relatively brief. Perhaps the optimal endocrine therapy would be one that induces near or total loss of the ER from the cell. This would circumvent the problems of cellular adaptation to low concentrations of estrogen and the intrinsic agonist activity of certain ligands. The pure steroidal antiestrogens are thus of great interest. Not only do they block both transcription-activating domains of the ER and have pure antiestrogenic activity on transcription of most, if not all, genes that have been studied, but they also induce loss of ER from the cell (Dauvois et al. 1992, Nicholson et al. 1996). Cultured cells, breast tumors from in vivo experimental model systems, and tumors from people, have been shown to have markedly lower ER levels after treatment with ICI 182,780.

Recent data from an experimental model also suggest that ICI 182,780 may have superior antitumor effects (Osborne et al. 1995). Tumor regression was greater and the duration of remission was twice as long with the pure antiestrogen as that seen with tamoxifen or estrogen deprivation in an athymic nude mouse model. Furthermore, tumors developing resistance to tamoxifen or to estrogen deprivation were sensitive to the pure antiestrogen, suggesting that these therapies have different mechanisms of resistance. A clinical trial in patients with metastatic breast cancer who were resistant to tamoxifen confirmed the high level of antitumor activity of this compound, and it is now in Phase III clinical trials comparing it directly with the aromatase inhibitor, anastrozole, and with tamoxifen itself (Howell et al. 1995). One might hypothesize that ICI 182,780, because of its pure antiestrogenic qualities and its effects in decreasing tumor ER, might induce more remissions in ER-positive tumors than other endocrine therapies, and that those remissions might be longer. It also seems likely, however, that tumors progressing after long-term treatment with pure antiestrogens will be totally estrogen independent. Overall survival, then, may not be affected. Additional study will be needed to determine the optimal endocrine therapy and the optimal sequence of available therapies.

Conclusions

Greater knowledge of ER function at the molecular level has provided new ideas on how endocrine therapies work and insight into possible mechanisms of hormonal resistance. It is clear that breast cancer cells can adapt, and subsequently be stimulated by very low estrogen concentrations after prolonged estrogen deprivation. Further reductions in estrogen concentrations by treatment with aromatase inhibitors is therefore helpful in some patients. The molecular mechanisms for this adaptation to
low estrogen concentrations is not known, but one possibility is alterations in the levels of receptor-interacting proteins that could increase ER sensitivity. Our new understanding of ER biology also offers the potential for developing the perfect drug with ideal mixtures of antioestrogenic and estrogenic activities, which would be extremely useful in breast cancer prevention or in hormone replacement therapy.

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