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Review paper

Acquired tamoxifen resistance in human breast cancer—potential mechanisms and clinical implications

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The widespread use of the antiestrogen tamoxifen in the management of breast cancer has resulted in more patients eventually developing acquired resistance to the drug. Tumors may often retain sensitivity to further endocrine therapies despite resistance to tamoxifen. The basis for this partial form of acquired resistance *in vivo* has been the subject of several recent investigations and the likely mechanisms are reveiwed in this article. Ineffective antiestrogen blockade could result from metabolic tolerance and inadequate intra-tumoral concentrations of the drug. Alternatively, there is experimental evidence that tamoxifen's partial agonist activity may be responsible for stimulation of tumor re-growth. Studies of the estrogen receptor (ER) have shown that in many cases expression of a fully functional wild-type receptor continues at relapse. Experimental evidence that mutant or variant forms of the receptor may account for resistance have not been confirmed by recent *in vivo* studies. There is some evidence for re-modeling of ER expression at relapse and it remains to be determined if there is enhanced sensitivity of ER+ cells to hormonal stimuli at relapse. Clonal selection of an ER− phenotype may occur in some instances, especially in patients with ER+ breast cancer who fail on adjuvant tamoxifen with relapse at distant sites. Finally, there is an increased understanding of the molecular pathways which regulate cell growth and apoptosis in hormone-sensitive cells and constitutive activation of these may provide the cell with a mechanism to bypass the requirement for estrogens. These advances in tumor biology have been matched by the clinical development of novel antiestrogens with less agonist activity and several clinical trials are ongoing to see if these new agents can delay the onset of acquired antiestrogen resistance.

Key words: Acquired resistance, breast cancer, estrogen receptor, tamoxifen.

Introduction

Tamoxifen is the most commonly prescribed drug for breast cancer in the world, and over two decades its role has expanded from primary treatment for advanced metastatic disease[1] to established adjuvant therapy following surgery for early breast cancer which prolongs both disease-free and overall suvival.[2] Tamoxifen is now being explored in clinical trials as a potential chemopreventive agent in women at high risk of developing breast cancer.[3] Not all patients with advanced breast cancer will respond to tamoxifen and of those who do most eventually acquire resistance to the drug. The widespread usage of tamoxifen in clinical practice has resulted in a significant increase in the number of patients presenting with tamoxifen-resistant breast cancer. The aim of this article is to review recent data on the likely mechanisms for acquired tamoxifen resistance which may operate *in vivo* and to discuss the development of various novel antiestrogens which ultimately may overcome this emerging clinical problem.

Tamoxifen resistance—clinical observations

Although tamoxifen will induce remissions in over half the patients with estrogen receptor (ER)+ metastatic breast cancer, and prolong both disease-free and overall survival in the adjuvant setting, eventually all patients develop resistance. In clinical practice two types are recognized: (i) intrinsic or *de novo* resistance where a tumor fails to respond to tamoxifen and the disease continues to progress despite antiestrogen therapy, and (ii) *acquired* resistance where the tumor responds initially before becoming resistant and regrowing. Clinical data suggest a significant difference in the overall survival of these groups of patients. In a series of 274 patients with advanced breast cancer treated with tamoxifen, 9% had a complete response with a median survival of 6 years, whereas those with

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either a partial response (21%) or stabilization of disease (20%) had a median survival of 3 years.[4] In contrast, the remaining 50% with *de novo* resistant progressive disease had a shorter median survival of only 14 months. Initial endocrine sensitivity may select a small group of patients with a relatively good prognosis, as confirmed recently by long-term follow-up of metastatic breast cancer patients treated with tamoxifen.[5] Second-line endocrine responses following tamoxifen failure suggest that many tumors have only *partial* endocrine resistance specific to tamoxifen, with retained endocrine sensitivity to further manoeuvres such as estrogen deprivation. Alternatively, tumors may have developed *complete* endocrine resistance, a phenotype which may also exist from the outset (i.e. *de novo* resistance) (Figure 1).

Patients who relapse during adjuvant tamoxifen therapy have also, by definition, disease which is resistant to tamoxifen. Although clinical data on primary response to tamoxifen are not available in such patients, two factors may help to determine the clinical type of resistance. Firstly, if the original tumor was ER+ it is more likely to have been endocrine sensitive at the outset and thus relapse during adjuvant tamoxifen therapy with acquired resistance. Secondly, a long disease-free interval following surgery may predict for endocrine sensitivity as recurrence within a few months is more likely in *de novo* resistant tumors. While there are no conclusive clinical data to support this statement, there is evidence that patients who stopped adjuvant tamoxifen for more than 2 years prior to relapse may benefit from tamoxifen re-challenge, implying that those with a long disease-free interval may still have endocrine-sensitive tumors.[6]

It is unlikely that one single mechanism can explain tamoxifen resistance in all breast cancer patients. Fundamental differences in tumor biology probably do exist between those with acquired tamoxifen-specific (i.e. partial) resistance compared with *de novo* (i.e. complete) endocrine resistance. Tumors with *de novo* resistance invariably lack a functional ER and may depend instead on alternative growth regulatory pathways. However, the biological basis for acquired tamoxifen resistance is less well understood. The pharmacological activity of tamoxifen and its metabolites together with the functional activity of the ER represent two important areas which have been examined recently. While experimental data exist to

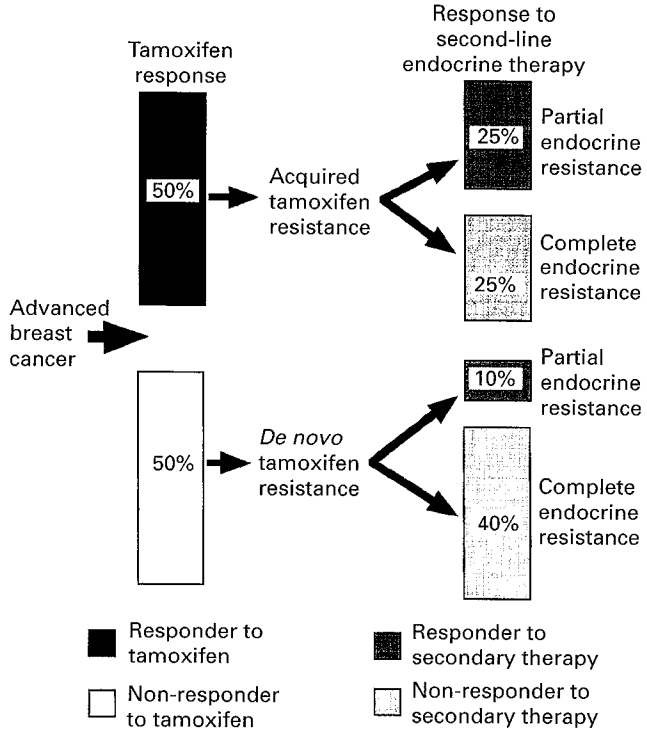

Figure 1. Response to second-line endocrine therapy after tamoxifen failure. Approximately 50% of patients with advanced breast cancer will respond to tamoxifen. When these patients relapse with *acquired* tamoxifen resistance, half (25% of original) will respond to further endocrine therapy and, thus, by definition, have only *partial* endocrine resistance. In contrast *complete* endocrine resistance may exist *de novo* in 40% patients who do not respond to tamoxifen or second-line therapy and in a further 25% who develop endocrine resistance after an initial response to tamoxifen. A small subgroup (10%) may respond to second-line therapy despite never responding to tamoxifen.

support the various hypotheses for acquired resistance outlined below, until recently there have been few applied clinical studies.

Endocrine adaptation

One potential mechanism for acquired resistance could be a change in the hormonal environment associated with treatment failure. Initial evidence suggested that patients who responded to aminogluthethimide therapy developed elevated circulating estrogen levels at the time of relapse.[7] In pre-menopausal women receiving continuous tamoxifen both estrogen and progesterone levels rise significantly above normal,[8] which theoretically could reverse tamoxifen's competitive antagonism. Furthermore elevated estradiol levels *in vivo* can reverse the growth inhibitory action of tamoxifen on MCF-7 xenografts in athymic mice.[9] However, in clinical practice any elevation in serum estradiol which may occur in pre-menopausal patients has not been associated with failure of tamoxifen therapy.

Tamoxifen-stimulated growth

The pharmacological properties of tamoxifen are complex as the drug can behave as an estrogen (full agonist), a partial agonist or as an antagonist. The specific effects of tamoxifen on human breast cancer have been studied in cell lines, particularly MCF-7 cells which contain estrogen receptors[10] and are growth stimulated by estradiol *in vitro*.[11] Tamoxifen inhibits MCF-7 cell growth,[11,12] and subsequent studies of cell cycle kinetics confirmed the cytostatic nature of tamoxifen which reduces the proportion of cells in S phase and increases the number of cells in G_1.[13,14] Numerous *in vitro* studies have investigated the molecular basis for acquired tamoxifen resistance using cell lines in which resistance has been induced through long-term culture.[15] However, these are of limited value when assessing the role of other associated factors such as drug pharmacokinetics, metabolism and stromal-epithelial interactions which may be relevant *in vivo*.

Animal model of acquired tamoxifen-resistance *in vivo*

MCF-7 xenografts established in ovariectomized nude mice and treated with long-term tamoxifen have become a useful model in which to investigate acquired antiestrogen resistance in human breast cancer. Previous studies have shown that while tamoxifen will cause partial regression of established xenografts,[16,17] continued administration is associated with acquired resistance and tumor re-growth.[18] Subsequent studies have shown these tumors to be growth-stimulated by tamoxifen in a dose-dependent manner and demonstrated that growth can be reduced by withdrawal of tamoxifen.[19]

It has been suggested that the acquisition of specific tamoxifen resistance may be associated with the agonist properties of the drug and/or its metabolites.[20,21] Inhibition of tamoxifen-stimulated growth in the MCF-7 xenograft model was originally demonstrated using a pure steroidal antiestrogen ICI 164,384, a steroidal antiestrogen compound with no inherent agonist activity.[19] In subsequent experiments when MCF-7 xenografts were treated from the outset, the pure antiestrogen ICI 182,780 significantly prolonged the time to tumor regrowth compared with tamoxifen.[22] If the basis for acquired resistance involved stimulation by tamoxifen, then it was hypothesized that estrogenic metabolites could contribute to tamoxifen's agonist activity at relapse. If so, structural analogs in which their formation is prevented or reduced may prove more effective antiestrogens. Idoxifene is a non-steroidal antiestrogen which is metabolically more stable than tamoxifen, with a preferable antagonist/agonist profile which inhibits hormone-dependent tumor growth *in vitro* and *in vivo* more effectively than tamoxifen.[23] There is clinical evidence that the drug may be active in some patients with advanced breast cancer who failed tamoxifen[24] and in MCF-7 xenografts equimolar doses of idoxifene inhibited tumor growth significantly more than tamoxifen (Figure 2).[25] Together with the data on ICI 182,780,[22] these experimental data provide evidence that more effective antiestrogens with reduced agonist activity may give greater inhibition of tumor growth which ultimately may retard the onset of acquired resistance.

Clinical evidence for tamoxifen stimulation

There are several anecdotal reports in the literature of patients with advanced breast cancer responding following simple withdrawal of tamoxifen, consistent with relapse in these patients being associated with stimulation by tamoxifen.[26] Two clinical trials have suggested the frequency of response to tamoxifen withdrawal to be 10–30%.[27,28] In the latter study 14 responses (22%) were from patients with 'no change' in tumor measurements for at least 6 months, a category which the authors have previously shown to give a similar duration of response and overall survival to those with an objective partial response.[4] The

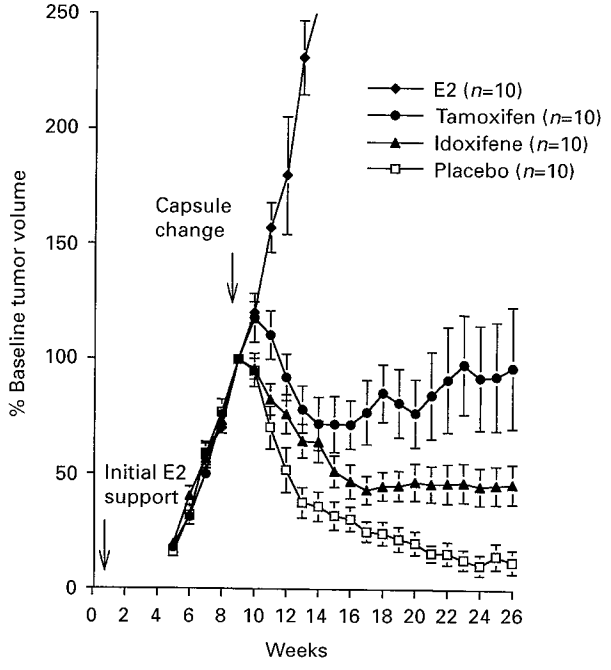

Figure 2. Effect of tamoxifen, idoxifene or estradiol withdrawal on growth of MCF-7 xenografts in nude mice.

median duration of response to tamoxifen withdrawal was 10 months and most responses were seen in soft tissue disease.

Additional evidence that tamoxifen may stimulate breast cancer at the time of relapse may be drawn from two small clinical trials which studied the response to oophorectomy after tamoxifen failure in pre-menopausal women. In patients who had previously responded to tamoxifen before developing acquired resistance, seven of 15 had an objective response to oophorectomy alone,[29] compared with none of 14 treated by oophorectomy plus continued tamoxifen.[30] These data would be compatible with continued tamoxifen stimulating breast cancer growth despite estrogen deprivation at the time of relapse.

Agonist activity of tamoxifen and its metabolites

The experimental and clinical data above have suggested that tumors may become specifically growth dependent on tamoxifen following continous exposure, which in some instances may be reversed by simple withdrawal of the drug. The biological basis for this specific form of acquired resistance remains unclear. It may represent a drug-specific form of resistance related to the inherent partial agonist activity of the drug or its metabolites as a consequence of systemic or intratumoral alterations in the drug's metabolism.

Tamoxifen's metabolism

The predominant metabolism of tamoxifen occurs in the liver via cytochrome P-450 enzymes located in the microsomes.[31,32] The two major metabolic pathways for tamoxifen are shown in Figure 3, and involve demethylation, deamination and hydroxylation of key positions on the phenyl groups. Demethylation of the tertiary amine results in the major metabolite found in human serum, *N*-desmethyltamoxifen.[33] The alternative route of metabolism involves hydroxylation of tamoxifen at the 4-position to from 4-hydroxytamoxifen, a potent antiestrogen.[34,35]

All the major metabolites of tamoxifen bind ER *in vitro* and competitively inhibit estradiol-stimulated growth of MCF-7 cells. However, there is a considerable range in the relative binding affinity (RBA) for ER of the different metabolites. In particular metabolites which are hydroxylated in the 4-position have a high RBA which is similar to estradiol[36] and are thus 100 times more potent than the parent compound tamoxifen in inhibiting MCF-7 growth *in vitro*.[12] It is thought that all the major metabolites contribute to tamoxifen's antiestrogenic activity *in vivo*, although some of the minor metabolites such as metabolite E and bisphenol appear to have significantly more agonist than antagonist properties in a variety of bioassay systems.[37] In addition metabolites of tamoxifen which are hydroxylated on the phenyl ring, i.e. 4-hydroxytamoxifen and metabolite E, are capable of undergoing time-temperature-dependent isomerization from the *Z* (*trans*) isomer to the *E* (*cis*) isomer.[38] These configurations may be important when discussing the relative estrogenic and antiestrogenic activities of tamoxifen's metabolites, e.g. *Z* (*trans*) 4-hydroxytamoxifen has a high RBA similar to that of estradiol and is a potent antiestrogen, whereas the *E* (*cis*) 4-hydroxytamoxifen has a low RBA and is a weaker antiestrogen.[39]

Evidence that an alteration in the metabolic profile of tamoxifen could account for acquired resistance came initially from the MCF-7 xenograft model where a significant increase in the *cis/trans*-4-hydroxytamoxifen ratio in tamoxifen-resistant versus tamoxifen-sensitive xenografts was noted.[21] The authors implied that the relative increase in *cis*-4-hydroxytamoxifen may be sufficient to induce a tamoxifen-stimulated response. Subsequently the same group reported finding high *cis/trans*-4-hydroxytamoxifen ratios in six tumors from patients with tamoxifen-resistant breast cancer.[40] In a further study of five patients with tamoxifen non-responsive breast cancer the same investigators identified the estrogenic metabolite E.[41]

Although these estrogenic metabolites were detected, it seems unlikely that their low concentrations

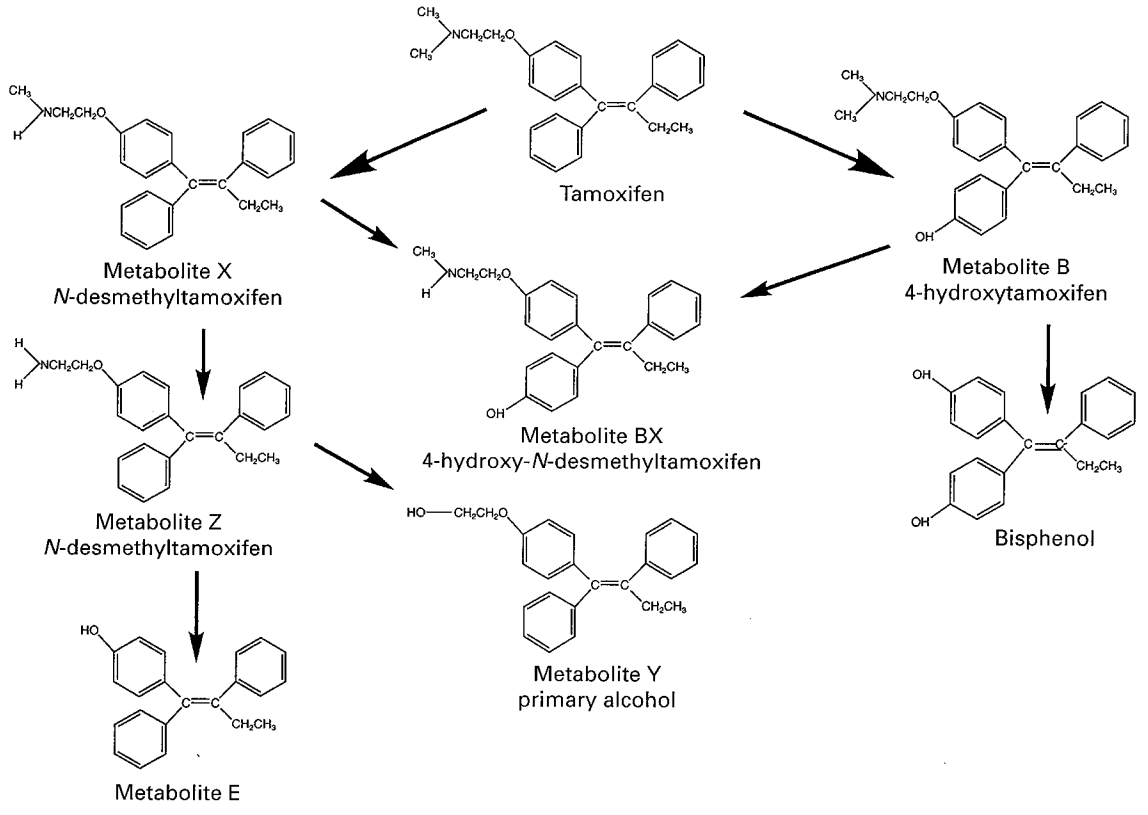

Figure 3. Metabolic pathway for tamoxifen.

could compete with the antiestrogenic levels of tamoxifen, *N*-desmethyltamoxifen or *trans*-4-hydroxy-tamoxifen which were present in these same tumors. Furthermore the contribution of isomerization as a mechanism for tamoxifen-stimulated growth *in vivo* has been questioned by some investigators. Fixed-ring analogs of tamoxifen have been synthesized in which isomerization is prevented.[42] These analogs stimulated the growth of the MCF-7 variant equally compared with the parent compound, implying that isomerization was not necesssary for tamoxifen-stimulated growth.[43] Furthermore analogs of tamoxifen which are resistant to conversion to metabolite E (deoxytamoxifen) also stimulated growth.[44] These experimental observations suggest that tamoxifen-stimulated growth, at least in the particular xenograft model studied, cannot be explained by isomerization or metabolism of tamoxifen to less antiestrogenic or more estrogenic metabolites. However, it remains possible that the resistant tumor is stimulated by the inherent partial agonist activity of the parent compound.

Metabolic tolerance

Metabolic tolerance has been used to describe a mechanism for resistance whereby reduced intra-cellular tamoxifen levels develop, either through enhanced metabolism or impaired intra-cellular accumulation.[21,44] Failure to achieve intra-tumoral levels sufficient to antagonize the interaction of estradiol with ER may allow hormone-dependent breast cancer cells to escape growth control by tamoxifen. Published *in vivo* data have suggested that rapid uptake of drug occurs resulting in intra-tumoral concentrations more than 10-fold greater than serum.[45-47] It has been debated the extent to which cellular proteins with a high affinity for tamoxifen, such as the ER, contribute to this intracellular accumulation[48] Alternative anti-estrogen binding sites (AEBS) within the cytosol have been described which may bind tamoxifen in a saturable fashion[49] and one study has suggested that enhanced AEBS activity could be one biochemical resistance mechanism to prevent tamoxifen's interaction with ER.[50]

Reduced intra-tumoral tamoxifen

While it has been shown that serum levels of tamoxifen remain constant during prolonged therapy *in vivo*,[51] there is both experimental and clinical evidence that reduced intra-tumoral accumulation may

occur following continuous tamoxifen exposure. In MCF-7 xenografts which had become tamoxifen-resistant following prolonged therapy a 10-fold reduction in intra-tumoral tamoxifen compared with responding tumors was observed in the absence of any change in the serum concentrations.[21] In a small clinical study from the same investigators reduced intra-tumoral tamoxifen concentrations were found in eigh of 11 'non-responsive tumors' treated for between 1 month and 6 years,[40] although serum tamoxifen levels were not measured to exclude poor compliance. Data from breast cancer patients with clinically defined tamoxifen resistance has confirmed that acquired, but not *de novo*, resistance to tamoxifen may be associated with reduced intra-tumoral levels in the presence of maintained serum levels (Figure 4).[52] This phenomenom was also observed in tumors which relapsed during adjuvant therapy, predominantly in patients who recurred following a longer disease-free interval.

Reduced intra-tumoral tamoxifen could result from a transport pump mechanism, such as overexpression of the 170 kDa P-glycoprotein coded for by the MDR 1 gene which is associated with reduced intra-cellular concentrations of various cytotoxic drugs.[53] Tamoxifen is a known substrate for P-glycoprotein[54] and in one study a 30% reduction in intra-cellular tamoxifen concentration was observed in a multidrug-resistant MDA-MB-A1 breast cancer cell line compared to control MDA-MB-231 cells which did not express P-glycoprotein.[55] In a separate study a significant increase in expression of P-glycoprotein was observed in tamoxifen-resistant tumors compared with tumors which responded to primary tamoxifen, although it was not possible to determine whether intra-tumoral levels were reduced in those tumors.[56] However, previous studies with tamoxifen-resistant xenografts have failed to find evidence for enhanced P-glycoprotein expression[57] and in a breast cancer cell line transfected with MDR 1 there was no evidence of reduced intra-tumoral tamoxifen concentrations.[58] These observations do not exclude a role for another active efflux pump such as the multidrug resistance associated protein (MRP), an ATP-binding drug transporter which is expressed in primary breast carcinomas, some of which do not express MDR 1 encoded P-glycoprotein.[59]

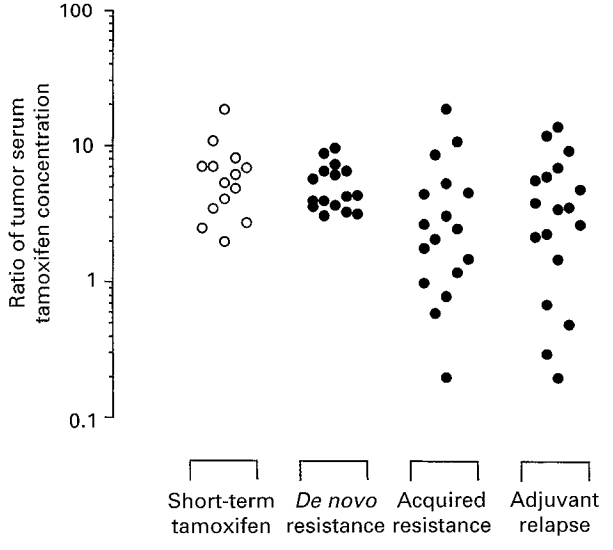

Figure 4. Intra-tumoral tamoxifen accumulation expressed as the ratio of tumor to serum concentrations (ng/g:ng/ml) in 51 patients with tamoxifen resistance and 14 short-term treated controls.

Stimulation by low dose tamoxifen

There is evidence that low tamoxifen concentrations in themselves may be growth stimulatory. Tamoxifen has been shown to stimulate the proliferation of T-47D human breast cancer cells at low concentrations (10^{-9} M) in the presence of charcoal-stripped fetal calf serum.[60] Likewise using the Courtenay-Mills clonogenic assay for MCF-7 cells in estrogen-free conditions, tamoxifen stimulated colony formation at low concentrations (10^{-9} to 10^{-11} M) but inhibited growth at much higher concentrations (10^{-6} M).[61] Furthermore, there is biochemical evidence that when tamoxifen therapy is first initiated progesterone receptor levels rise, a response normally considered to be estrogenic.[62]

Antiestrogen sensitivity

An alternative explanation for tamoxifen-stimulated growth in patients with acquired resistance may involve alterations in the estrogen response pathway whereby individual cells respond to tamoxifen by being growth-stimulated rather than growth-inhibited. This could occur if, within a heterogenous endocrine-sensitive tumor, clones of cells exist with an altered sensitivity to tamoxifen.[20] Clonal selection of cells with differential expression of progesterone receptor (PgR) has been demonstrated by flow cytometry to occur in breast cancer cell lines treated with tamoxifen.[63] Re-modeling of a heterogenous tumor, therefore, is a theoretical possibility which may explain not only tamoxifen-stimulated growth, but also tamoxifen withdrawal response followed by a subsequent second endocrine response (Figure 5).

The molecular explanation for cells perceiving tamoxifen as a growth stimulus may relate to changes

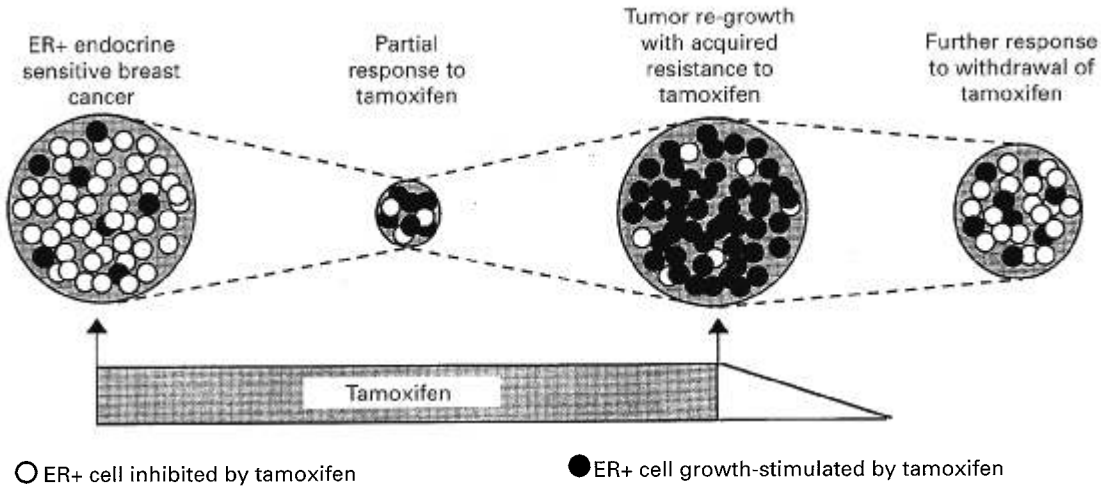

Figure 5. Model of acquired tamoxifen resistance due to clonal selection of ER+ cells which are growth-stimulated by tamoxifen. While the original tumor may be hetereogenous in terms of cells with different inherent sensitivity to tamoxifen, initial therapy with tamoxifen will eliminate hormone-sensitive cells and favor the selective outgrowth of cells which are growth-stimulated by tamoxifen. This will allow the emergence of an ER+ tumor which perceives tamoxifen as a growth stimulus. Further endocrine responses may be achieved either by withdrawal of the 'stimulatory' tamoxifen or by use of a second-line endocrine agent with no agonist activity. Thus, the ER+ growth-stimulated cells would remain sensitive to effective blockade or deprivation of hormonal signals.

in ER which alter the downstream response to the drug-receptor complex. Point mutations generated by site-directed mutagenesis have shown that amino acids in the ligand-binding domain of ER may be crucially important in determining the response to various ligands. Transfection of either wild-type or point-mutated human ER into ER− MDA-MB-231 breast cancer cells altered the response such that cells became growth inhibited by estradiol,[64] yet demonstrated enhanced agonist activity in response to 4-hydroxytamoxifen.[65] Such a point mutation may determine the critical folding of the ligand-binding domain into a conformation which allows ER to recognize various ligands as agonists or antagonists.[66] Thus although various ligands may determine the outcome of interaction with wild-type receptor,[67] a different conformation of mutated receptor with a particular ligand may also generate an altered cellular response.

ER expression

There is considerable clinical evidence that ER expression is the major determinant of primary endocrine responsivenes in untreated breast cancer.[68] In postmenopausal patients approximately 60% of tumors contain ER and in these an objective response rate of up to 70% may be expected with endocrine therapy. In contrast, tumors without significant quantities of ER have only a 5–10% chance of responding to endocrine therapy. However, breast carcinomas are well known to be heterogenous in terms of hormone sensitivity and expression of ER/PgR.[69] Prolonged estrogen deprivation of hormone-dependent breast cancer cells *in vitro* is known to select a population of cells which grow independently of estrogen and are refractory to the inhibitory action of antiestrogens.[70] The question which has arisen in patients with ER+ breast cancer, however, is whether prolonged tamoxifen modulates ER expression and function, such that selection of ER− cells occurs and alters both tumor phenotype and endocrine responsiveness.

Clonal selection and re-modeling

It was first reported that a significant fall in ER content followed endocrine therapy,[71–73] implying that tamoxifen may modify ER expression and favor the emergence of ER− tumors. It has since been shown that in tumors treated with tamoxifen, ER is detected more frequently by immuno-histochemistry compared with ligand-binding assay,[74] suggesting that in earlier reports receptor occupancy by tamoxifen could have resulted in a false negative ER by ligand-binding assay in some tumors.

Clonal selection is an attractive explanation for the development of tamoxifen resistance *in vivo*. The clinical observation that many patients who develop acquired resistance to tamoxifen remain sensitive to further endocrine therapies, including pure antiestrogens[75] and aromatase inhibitors,[76] suggests that ER may be expressed and functional in many tumors with

acquired tamoxifen resistance. In a series of 72 breast cancer patients treated with tamoxifen, we found that 61% of previous responders remained ER+ at relapse whereas all non-responders were ER−.[77] These data are consistent with half of the tamoxifen responders having only partial endocrine resistance at relapse (Figure 1). Other groups have reported retention of ER in a series of asynchronous breast recurrences which predicted for response to further endocrine maneuvers.[78] We also found a significant number of *de novo* resistant tumors which expressed PgR/pS2 at progression in the absence of any immunoreactive ER.[77] While previous studies have attributed this ER−/PgR+ phenotype in tamoxifen-treated tumors to a false negative ER assay,[79] a similar observation was noted by Osborne's group using an immuno-histochemical assay with three different monoclonal antibodies.[74] In contrast we found that the majority of tumors which recurred during adjuvant therapy, many of which represented regional or metastatic recurrences, were ER− PgR/pS2−. Other studies have reported a similar drift towards a complete ER− phenotype in secondary sites of disease compared with the primary tumor,[78,79] and this may correlate with a metastatic phenotype which survives surgery and is clonally selected for during adjuvant tamoxifen therapy.

Despite retention of a functional ER with acquired tamoxifen resistance, a quantitative and qualitative change in ER expression may be observed in many tumors (Figure 6). Re-modeling of ER expression during prolonged tamoxifen therapy probably does occur to some extent. In situations where ER expression and function appears normal, it is unclear whether ineffective blockade by tamoxifen (i.e. because of metabolic tolerance) or selection of ER+ cells which are stimulated by tamoxifen (Figure 5) accounts for tumor re-growth. In either situation, tumors may remain sensitive to other therapies such as aromatase inhibitors. Alternatively, selection of cells with specific changes within either the receptor molecule (i.e. mutations or variant forms of ER) or its response pathway may be a principle mechanism for the development of acquired resistance, a situation which may render an ER+ tumor completely endocrine resistant (Figure 1).

Mutant or variant ER

Activation of the downstream molecular events involved in the estrogen/ER regulated pathway may facilitate hormonal escape. The ER protein functions as a ligand-modulated transcription factor which can be activated by estrogen to bind to specific enhancer sequences of DNA, termed estrogen response elements (EREs), adjacent to target genes.[80] One consequence of activated ER binding to DNA is a mitogenic signal to the cell, possibly through the release of local peptide growth factors such as transforming growth factor (TGF)-α.[81,82] Any structural alteration in ER may cause an uncoupling of the protein's ligand activation from its DNA-binding function and growth regulatory activity, and result in a non-functional transcriptionally inactive ER. Alternatively, an unconstrained ER may exist which is transcriptionally active independent of ligand. Thus mutated or structurally variant forms of ER may function as types of 'oncogene' with over-expression rendering a tumor hormone resistant.[83]

ER DNA mutations

Unlike many other oncogenes there is no evidence for ER gene amplification or re-arrangement in breast

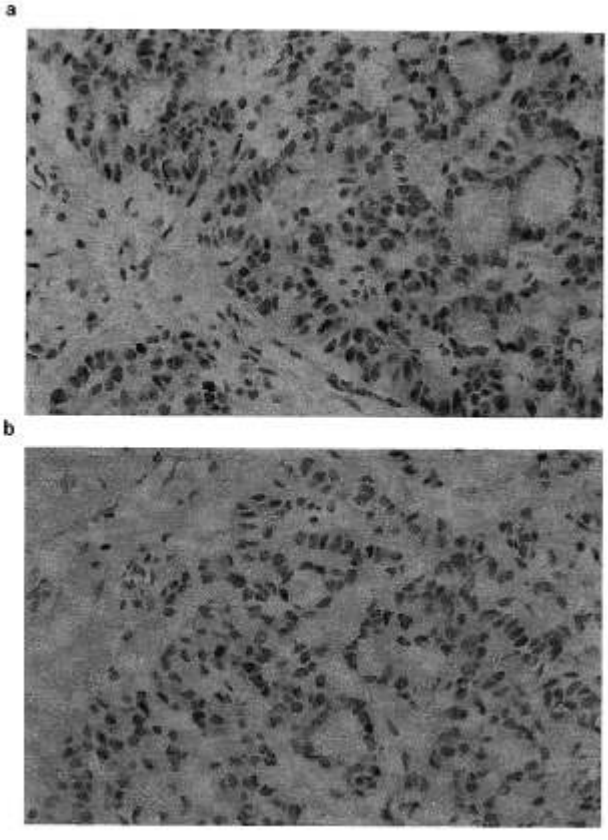

Figure 6. Immunohistochemical expression of ER in (a) primary untreated breast carcinoma and (b) tamoxifen-resistant locally recurrent breast carcinoma from the same patient at relapse following at least 2 years of adjuvant tamoxifen. The primary tumor was ER+ with an H-score of 160, while the tamoxifen resistant recurrence was still ER+, expression was more heterogenous with a lower H-score of 67. This was related to a marked reduction in the percentage of ER+ cells without any change in staining intensity (×400 original magnification).

cancer to account for aberrant ER function.[84] Point mutations in specific regions of ER generated by site-directed mutagenesis *in vitro* can significantly alter the pharmacological response of ER to tamoxifen from that of an antagonist to a full agonist.[85] A point mutation in ER's ligand-binding domain has been found in a tamoxifen-stimulated MCF-7 xenograft, accounting for 80–90% of the total ER and representing the first identification of a mutant ER protein from a transplantable tamoxifen-stimulated human tumor line.[86] When transfected into MDA-MB-231 cells, the mutant ER responded to 4-hydroxytamoxifen in a similar manner to estradiol.[87] While these experimental data imply a possible role for ER mutants in rendering a tumor 'resistant' to tamoxifen, there is no evidence for these critical mutations in human breast cancer *in vivo*.[88–90]

ER mRNA splice variants

During transcription of the ER gene and synthesis of its mRNA, alternative splicing with deletions of whole exons can occur which generates shorter mRNA transcripts. A variety of different ER mRNA splice variants involving precise deletions of whole exons (2, 3, 4, 5, 6 or 7) have been identified in human breast tumors.[91–95] The location of the splicing junctions flanking each exon determines whether the resulting spliced transcript is either in-frame, or instead results in a frameshift whereby a new codon reading sequence is introduced (Figure 7).

The variant receptors formed by translation of alternatively spliced mRNA may have a range of different properties within the cell.[96] The ER variant may be completely inactive, as occurs with the exon 2 deletion variant (ERΔE2) where a frameshift results in premature termination of translation giving a transcriptionally inactive protein.[95] ER variants may behave as a 'dominant negative' receptor capable of inhibiting the transcriptional activity of the wild-type receptor as occurs following in-frame deletion of exon 3.[97] Alternatively variant mRNA transcripts may code for a 'dominant-positive' receptor which becomes constitutively active independent of ligand, as exemplified by the ERΔE5 variant which has been shown *in vitro* to be transcriptionally active independent of estradiol.[91]

The significance of ER mRNA splice variants in relation to tamoxifen resistance has been the subject of several recent studies. Transfection of the ERΔE5 variant into MCF-7 cells confered a tamoxifen-resistant phenotype *in vitro*,[98] although others have shown that inducible expression of ERΔE5 in MCF-7 cells failed to prevent the growth inhibitory effects

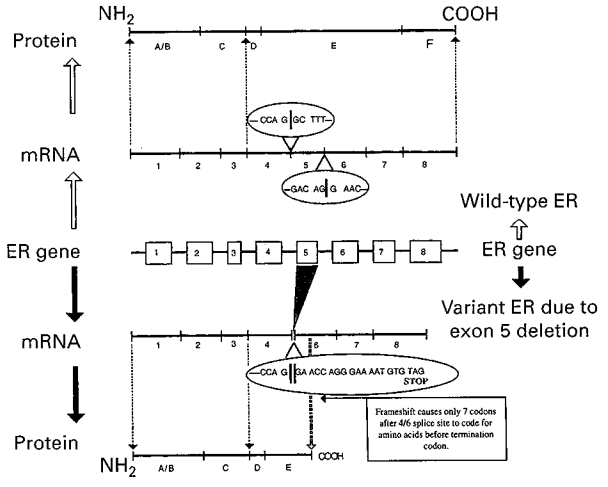

Figure 7. Transcription of ER gene with splicing of introns to form mRNA followed by translation of mRNA into ER protein. Numbers 1–8 refer to the eight known exons of the gene, which in turn code for the six functional domains of the ER protein (A–F). Domain C is involved with DNA binding, while domain E contains the ligand-binding domain. Splicing of whole exon 5 results in a frameshift in codon reading sequence of exon 4 into exon 6, with a truncated ER protein of approximately 40 kDa which lacks a major part of the ligand-binding domain, but which can still bind DNA via domain C.

of tamoxifen or ICI 182,780.[99] MCF-7 cells in which resistance to tamoxifen was induced through long-term culture (MCF-7/TAM^R-1) remained ER+ for both protein and RNA. Although a number of mRNA splice variants including deletions of exons 2, 4, 5 and 7 were found, semi-quantitative RT-PCR showed that the ERΔE5 variant was actually decreased in resistant cells.[100] In human breast carcinomas no difference was found in expression of ERΔE5 between tamoxifen-resistant and untreated tumors.[101] Taken together these recent data imply that acquired tamoxifen resistance is not always related to overexpression of this particular constitutively active ER variant.

Altered regulation of cell proliferation and cell death

Ultimately any understanding of the molecular and cellular basis for acquired hormone resistance is likely to require a knowledge of the mechanisms involved in cell proliferation and regulation of the cell cycle. While the importance of estrogen to the growth of many breast cancers is well established, one explanation for the acquisition of steroid insensitive growth could be the de-regulated expression of downsteam growth-regulatory pathways which release the cell cycle from normal steroid control.

Cell proliferation

Several genes are induced in the early part of G_1 phase, including the proto-oncogenes c-*myc* and c-*fos* whose expression is increased within 2 h of estrogen treatment of breast cancer cells *in vitro*.[102] Other cell cycle genes which are expressed include the cyclins and cyclin-dependent kinases (CDKs), molecules which form the regulatory and catalytic subunits, respectively, of cell cycle regulated kinases. The sequential transcriptional activation of cyclins and formation of specific cyclin/CDK complexes forms the basis of several defined control points within the cell cycle.[103] In particular cyclin D1 is the first G_1 phase cyclin to be induced following mitogenic stimulation with steroids and its level correlates with the rate of cell cycle progression.[104] During this phase antiestrogens reduce cyclin D1 expression prior to any decline in cell DNA synthesis, suggesting a causative role for this regulatory protein in mediating steroid-induced growth signals. However, recent evidence has suggested that cyclin D1 may activate ER-mediated gene transcription directly, independent of CDKs and also in the absence of estrogen.[105] This cyclin D1 action may allow cells to bypass the requirement for estrogens and provide another molecular mechanism which may account for steroid-independent growth in cyclin D1 overexpressing ER+ breast carcinomas[106].

Tamoxifen's anti-proliferative mechanism of action may also be mediated by alternative non-ER pathways including suppression of circulating levels of stimulatory peptide growth factors such as insulin-like growth factor-1,[107] inhibition of calmodulin which acts as a regulator of intra-cellular calcium pools,[108] inactivation of protein kinase C which is a cellular enzyme regulating various signals for cellular proliferation,[109] together with potential anti-angiogeneic properties mediated via inhibition of vascular endothelial growth factor expression.[110] Whether constitutive activation of any of these pathways is sufficient to account for steroid-independent growth *in vitro* or whether over expression occurs in tamoxifen-resistant breast carcinomas *in vivo* is unknown at present.

A reduction in mitotic index in MCF-7 xenografts treated with tamoxifen[13] and an accumulation in G_1 phase as determined by flow cytometry have been correlated with drug-induced growth inhibition in these animal models.[111] In clinical studies tamoxifen administered prior to surgery significantly reduced Ki-67 labeling index in ER+, but not ER−, primary breast carcinomas.[112,113] In a separate experimental study no difference was seen in labeling index or cell cycle distribution between MCF-7 xenografts treated with tamoxifen compared with estradiol, despite significant tumor regression.[114] These data suggested that tamoxifen may also exert an additional direct effect on inducing cell death rather than just slowing cell proliferation.

Programmed cell death—apoptosis

The cellular response to several anticancer compounds includes the induction of apoptosis, and cells which accumulate significant DNA damage and cannot be repaired are deleted by this process.[115] It follows that any defect in the apoptotic process (or the genes which regulate it) may result in cell survival rather than cell death and could represent a molecular mechanism for resistance to anticancer therapy. Apoptosis has been demonstrated in response to tamoxifen in ER+ cells *in vitro*[116] and as xenografts *in vivo* following treatment with the antiestrogen toremifene,[117] while studies have implicated TGF-β1 in the process of hormonally-induced apoptosis.[118] If the genes which regulate this apoptotic process become de-regulated this could account for resistance to therapy due to failure to undergo cell death. One such gene which may have a central role in regulating apoptosis is *Bcl-2*.

Bcl-2: a unifying mechanism for chemo-endocrine resistance

There has been much speculation that Bcl-2 and its family of related proteins may play a central role in drug resistance mechanisms in breast cancer. De-regulated expression of some of these proteins has the potential to prevent the cell's normal apoptotic response to cytotoxic agents. There is experimental evidence to suggest that Bcl-2 may be hormonally regulated,[119] together with clinical data which show a clear association of Bcl-2 with ER in human breast cancer.[120–122] Modulation of Bcl-2 expression by either endocrine or molecular means has already been shown to enhance chemo-sensitivity in breast cancer cells *in vitro*.[123,124] Recent evidence has suggested that ER+ c-*erbB2*$^+$ breast cancer cells have elevated levels of Bcl-2 and Bcl-x_L protein and are resistant to tamoxifen-induced apoptosis.[125] These preliminary data would support a role for de-regulated Bcl-2 conferring resistance to tamoxifen, although initial studies *in vivo* have suggested there is no significant change in Bcl-2 expression.[126] If anti-apoptotic mechanisms such as enhanced Bcl-2 expression exist in tamoxifen-resistant tumors, then similar molecular approaches to antagonize Bcl-2 expression or function could be envisaged clinically to circumvent this form of hormonal resistance.

Growth factor pathways

Estrogen causes indirect stimulation of cell replication through both increased release of peptide stimulatory growth factors and decreased secretion of growth suppressive factors.[81] These growth factors may act either as paracrine or autocrine signals to both ER+ and ER− in the tumor. Tamoxifen may function through competitive inhibition of estrogen binding which blocks these ER-mediated growth signals. Theoretically, therefore, escape from the tamoxifen control may occur through a variety of mechanisms including constitutive production of stimulatory growth factors, repression or inactivation of inhibitory growth factors, or overexpresion of growth factor receptors.

Growth stimulating peptide regulatory factors

TGF-α can act as an agonist ligand for the epidermal growth factor receptor (EGFr)[127] and has been detected in media conditioned from the MCF-7 breast cancer cell line.[128] In some hormone-independent cell lines constitutive expression of TGF-αa mRNA and EGFr mRNA is very high,[129] suggesting that unregulated autocrine cell growth may result if over expression of these growth factors occurs. Likewise, IGF-1 is a potent mitogen for breast cancer cells *in vitro* which may have paracrine and endocrine functions *in vivo*.[130] In patients with breast cancer tamoxifen induces a significant reduction in serum IGF-1 suggesting that part of tamoxifen's anti-tumor action may involve suppression of this circulating mitogen,[107] although other investigators have shown that tamoxifen directly reduces IGF-1 binding sites on tumor cells.[131] These observations raise the possibility that tamoxifen can interfere with the initial steps of growth factor binding and activity in both ER+ and ER− breast cancer cells. In acquired tamoxifen resistance, therefore, IGF-1 could function as a growth promoter if levels became elevated during therapy.[132] However, these individual stimulatory growth factors (even if over expressed) are incapable of replacing estrogen as an essential requirement for tumor growth in animal models.[133] Furthermore, not all estrogen-independent cell lines that have been developed show evidence of enhanced growth factor expression. For example, T47D-C4 cells (ER/PgR) have a low level of expression of both TGF-α and EGFr, yet will grow independent of estrogen.[134] There may be many other peptide growth factors, including fibroblast growth factors and others yet to be identified, which may become involved in the development hormone resistance.

Growth inhibitory peptide regulatory factors

TGF-β1, a known inhibitory growth factor for breast cancer cells *in vitro*,[135] may be another important mediator for tamoxifen's anti-tumor action. Tamoxifen induces TGF-β1 mRNA production from cultured estrogen-stimulated breast cancer cells and in addition may induce secretion of TGF-β1 from fetal human fibroblasts *in vitro*.[136] TGF-β1 is thought also to promote stromal cell growth, enhance accumulation of extra-cellular matrix, and act as a macrophage chemotactic factor and local immunosuppressive agent, all of which may contribute to growth inhibition *in vivo*. Down-regulation of these growth inhibitory pathways could be a possible mechanism for escaping tamoxifen control. Preliminary evidence suggested that some hormone-unresponsive cell lines may lose their sensitivity to exogenous TGFβ with a reduction in the extent of growth inhibition observed,[137] while data from a clinical study showed that TGFβ1 mRNA expression in breast cancer patients was enhanced in patients whose tumors progressed whilst on tamoxifen.[138] It becomes clear that it is difficult to explain autonomous growth on the basis of aberrant expression of one single growth factor.

Growth factor receptors

Growth factor receptors mediate the growth signal of peptide growth factors such as TGF-α. The overexpression of both EGFr and c-*erbB2* has been studied extensively in breast cancer, and there is evidence that they may represent markers of estrogen-independent growth control. EGFr, a 75 kDa protein coded for by the c-*erbB1* oncogene on the short arm of chromosome 7,[139] is expressed at a high level in 20% of breast carcinomas. Expression of EGFr is inversely correlated with ER[140] and clinically this has been shown to predict for a poor prognosis.[141] The c-*erbB2* oncogene on the long arm of chromosome 17 codes for a 138 kDa transmembrane protein which shows structural similarity to EGFr with an extracellular ligand-binding domain and intracellular tyrosine kinase activity.[142] Frequent amplification of the c-*erbB2* oncogene has been demonstrated in both breast cancer cell lines and human breast cancer tissue.[143] Likewise amplification of this cellular proto-oncogene correlates with a poor prognosis in human breast cancer in terms of survival, time to relapse and positive lymph node status,[144] and it has been suggested recently that c-*erbB2* overexpression may be associated with a lack of response to endocrine therapy.[145]

A molecular basis for tamoxifen resistance

Several groups have adopted the approach of generating acquired hormone-resistance cell lines *in vitro* with the hope of isolating and cloning new genes which may be responsible for the development of resistance.[146] Ultimately this may allow identification of some of the molecular events downstream of ER which may be involved in the step-wise progression of breast cancer towards hormone independence.

ER's transcriptional activity

Specific proteins associate with the ligand/ER/ERE complex to regulate transcriptional activation of estrogen responsive genes, and the relative amounts of these 'co-activator' or 'co-repressor' transcription factors may determine ligand-inducible gene transcription within a promoter and cell-specific fashion. Several identifed co-activators mediate estradiol's agonist effects including RIP140[147] and SRC-1/ERAP-160.[148] It remains unclear whether levels of co-activator protein

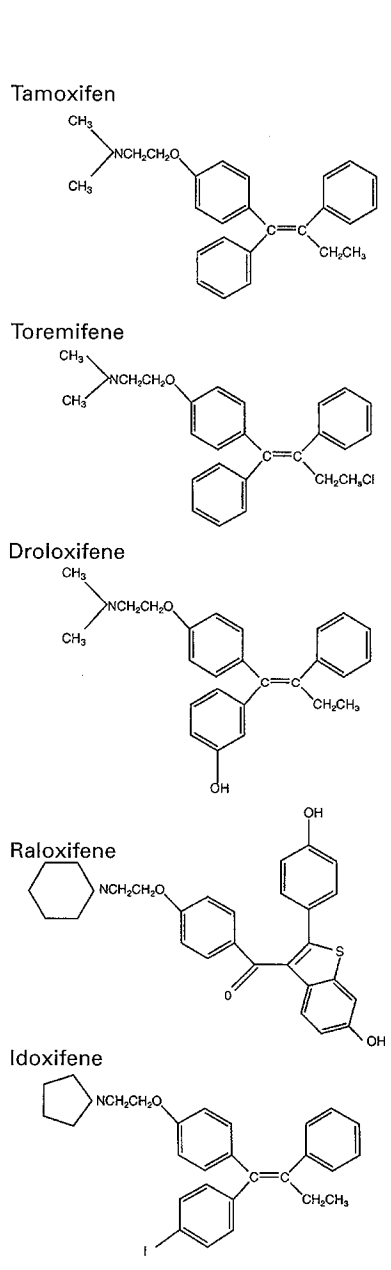

Figure 8. Structures, relative binding affinities for ER and percent agonism (in the absence of estradiol) of the five non-steroidal antiestrogens available clinically or in clinical development.

change with acquired resistance or whether alterations in distinct transcription factor pools are sufficient to account for an agonist response to tamoxifen. Several groups are investigating this aspect at present.

ER's transcriptional activity within individual cells may be determined by the promoter context, which in turn may dictate whether tamoxifen acts predominantly as an antagonist or as an agonist.[149] A model has been proposed to explain the tissue-specific partial agonist effects of tamoxifen, based on the different activities of ER's two transcriptional activation domains (TAF1 and TAF2) following binding of either estrogen or tamoxifen.[150] In certain cells and on certain promoters the functional activity of TAF2 may be provided independent of ER, allowing tamoxifen to generate an agonist signal through TAF1. Such a mechanism could account for the emergence of tamoxifen-stimulated ER+ tumors, but remains unproven.

Finally, evidence is emerging that ER may activate gene transcription independent of classical ERE-regulated pathways through the AP-1 pathway. ER has been shown to interact with Jun/Fos proteins at the AP-promoter site of certain genes, including the collagenase gene.[151] Furthermore tamoxifen was shown to act as an agonist through AP-1 in a tissue-specific manner, thus paralleling tamoxifen agonism *in vivo*. MCF-7 cells which acquired resistance to tamoxifen *in vitro* have increased AP-1 DNA binding[152] and a recent report has confirmed elevated AP-1 DNA binding in human tamoxifen-resistant tumors.[153] Whether this relates to increased levels of Jun or Fos proteins, or enhanced kinase activity of the complex remains to be determined. However, this alternative pathway for ER-mediated gene transcription may be relevant to the stimuatory response observed with tamoxifen in acquired resistance.

Role of novel antiestrogens as endocrine treatment of breast cancer

As a competitive antagonist tamoxifen is the most effective and best tolerated endocrine treatment which is considered first-line endocrine therapy for post-menopausal breast cancer. In light of our current understanding of the mechanisms of acquired tamoxifen resistance, what potential role do novel antiestrogens have in the clinic?

Treatment of advanced breast cancer

Non-steroidal antiestrogens with less agonist activity than tamoxifen are in clinical development including raloxifene,[154] idoxifene,[23] droloxifene[155] and toremifene.[156] The structure, relative binding affinity for ER and inherent agonist activity of these compounds in comparison with tamoxifen is shown in Figure 8. In addition, a pure steroidal antiestrogen (ICI 182,780) has entered clinical development having shown complete absence of any agonist activity in pre-clinical studies.[157] Evidence suggests that the ligand-receptor conformation induced by some of these compounds may impart a different transcriptional response within the cell in comparison with the tamoxifen-ER complex. In particular the agonist activity of ER, normally mediated by tamoxifen through TAF-1-dependent gene transcription, was prevented by both raloxifene and the pure antiestrogen ICI 164,384.[167] Furthermore, experimental evidence exists that while breast carcinoma cells established in clonogenic assay from patients who relapsed on tamoxifen can be stimulated *in vitro* by either estrogen or 4-hydroxytamoxifen, they remain completely inhibited by ICI 182,780.[61] Additional studies are needed to determine whether the agonist basis for acquired tamoxifen resistance can be overcome by the non-steroidal antiestrogens.

The MCF-7 xenograft data imply that *in vivo* more effective estrogen antagonism may delay the emergence of acquired resistance.[22,25] In clinical practice this could translate into a significant benefit for breast cancer patients by prolonging the time to disease progression. It can be debated whether these novel antiestrogens should be used as primary therapy for metastatic breast cancer instead of tamoxifen or reserved for second-line therapy after tamoxifen failure. Xenografts which relapse following ICI 182,780, albeit much later than with tamoxifen, are associated with an ER-depleted hormone-independent phenotype.[25] Thus, although primary therapy with a novel antiestrogen can prolong time to disease progression, it may also induce complete endocrine resistance and remove the option for successful second-line endocrine therapy. In contrast patients with acquired tamoxifen resistance had a high response rate (greater than 50%) when treatment was changed to ICI 182,780.[75] Likewise, in phase I/II clinical trials after tamoxifen failure, response rates of 35–38% have been reported with the novel non-steroidal antiestrogens droloxifene[158] and idoxifene.[24] This implies that these agents may have a degree of non-cross-resistance with tamoxifen, especially if the basis for resistance is an agonist response to tamoxifen. Sequential therapy with novel agents after tamoxifen, therefore, may be an alternative strategy to maintain endocrine control for longer in patients with metastatic breast cancer.

Alternating endocrine therapy in advanced disease—a potential novel strategy

A breast cancer cell's sensitivity to various hormones may change as an adaptive mechanism to either prolonged exposure or deprivation. In a recent report MCF-7 cells deprived of estradiol for 1-6 months enhanced their sensitivity to estradiol, being maximally stimulated at 10^{-14} M in contrast to 10^{-10} M for wild-type cells.[159] A shift towards the left of the bell-shape dose-response curve could explain some secondary endocrine responses in breast cancer. For example, in pre-menopausal women with advanced breast cancer who respond to oophorectomy (with estradiol levels falling from approximately 400 to 30 pmol/l), a secondary response can be achieved at relapse using further estrogen deprivation achieved by aromatase inhibitors (where estradiol levels fall to below 10 pmol/l). This sequential approach to endocrine therapy, in particular step-wise estrogen deprivation, has proved effective with 4-hydroxyandostenedione following failure both with aminoglutethimide in post-menopausal women[160] and gonadotrophin releasing hormone agonists in pre-menopausal women.[161]

As a partial estrogen, tamoxifen has a similar bell-shaped dose-response curve *in vitro*.[60] Thus, continuous exposure to tamoxifen may equally alter the cell's responsiveness to the drug, such that at relapse a given concentration of tamoxifen may become growth stimulatory. Thus a shift in the cell's dose-response curve could explain not only the clinical response to tamoxifen withdrawal,[28] but also response to increased doses of tamoxifen[162,163] (Figure 9). Likewise this may provide a unifying hypothesis for the *in vivo* biological data which indicate maintained ER expression and function associated with metabolic tolerance of tamoxifen.[52,77] In response to this, a new endocrine strategy could emerge comprising either alternating hormonal therapies, or on/off periods of treatment with conventional agents such as tamoxifen or aromatase inhibitors. This would take advantage of these drug's different modes of action, and if sequenced correctly could prevent clonal selection and adaptive mechanisms by the tumor from inducing endocrine failure. Clinical trials will be required to see if this approach can provide more effective endocrine control and prolonged progression-free survival in patients with metastatic breast cancer.

Adjuvant endocrine therapy

The effectiveness of adjuvant tamoxifen in both node-positive and node-negative breast cancer was established in the world overview of 30 000 women from 133 randomized controlled clinical trials.[2] The optimal duration of adjuvant tamoxifen therapy in breast cancer has been the cause of much recent debate. A significant improvement in disease-free and overall survival was found in patients treated with tamoxifen for 5 years compared with 2 years,[164] whereas tamoxifen for more than 5 years was of no further benefit and could be detrimental.[165] The same biological explanation for acquired tamoxifen resistance in ER+ tumors (i.e. agonist stimulated growth) could account for these adjuvant data. While in metastatic disease tamoxifen may induce a response subsequent to tamoxifen-stimulated tumor regrowth after 1-2 years, in the adjuvant setting tamoxifen may maintain remission for 4-6 years prior to relapse by the same mechanism. The longer time interval reflects smaller tumor bulk when commencing tamoxifen (i.e. micro-metastatic versus advanced disease).

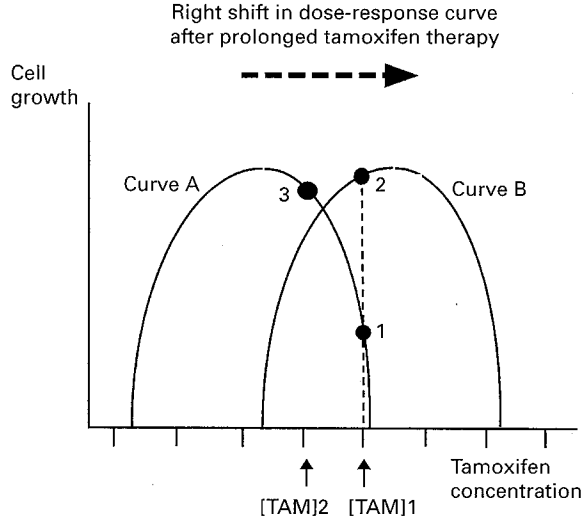

Figure 9. Potential shift in dose–response curve during prolonged tamoxifen therapy as an explanation for tamoxifen's agonist activity in acquired resistant tumors. In hormone sensitive cells, curve A may represent the normal dose–response to various concentrations of tamoxifen. For a physiological level of tamoxifen, i.e. [TAM]1, breast cancer growth in ER+ cells may be inhibited (point 1). A subsequent change after prolonged therapy with tamoxifen stimulation could occur in one of two ways—if there were a shift in the sensitivity of the cells manifest as a right shift in the curve (i.e. cells becoming supersensitive to the parial agonist activity of the antiestrogen, curve B), then the same concentration of tamoxifen [TAM]2 would become growth simulatory (point 2). Alternatively, in the absence of any change in the dose–response curve, a 10-fold lower level of tamoxifen to [TAM]2 within the tumor, as observed in some tamoxifen-resistant human tumors, could become growth stimulatory (point 3).

Table 1. Summary of potential mechanisms for acquired tamoxifen resistance in human breast cancer and the clinical implications

ER status at replapse	Resistance mechanism	Clinical comment
ER+	Metabolic tolerance (by drug exclusion or sequestration) in hormone-sensitive cells	In theory could respond to increased doses of tamoxifen, although evidence for this is scanty
	Stimulation of ER+ cells by agonist component of tamoxifen or its metabolites	Novel antiestrogens with less agonist activity (i.e. ICI 182,780 or idoxifene) may remain active
	ER mutants or variants which are either constitutively active (dominant positive) or inactive (dominant negative)	Little supportive evidence that ER mutants play a major role in clinical resistance
	Re-modeling of tumor with clones of ER+ cells with an altered sensitivity/response to tamoxifen	May remain sensitive to further endocrine therapies (i.e. aromatase inhibitors)
	Activation of ER-regulated growth pathways independent of steroid control (e.g. cyclin D1; AP-1)	Tumors likely to be completely endocrine resistant
ER−	Clonal selection of ER− cells from an original ER+ tumor	More likely in metastatic recurrences after adjuvant tamoxifen
	Overexpression of growth factors or their receptors	Likely that tumors would have developed complete endocrine resistance

These data are important when considering the potential role of novel endocrine therapies in delaying relapse in the adjuvant setting. Unlike patients with advanced breast cancer, a significant survival benefit for patients with early breast cancer may occur as any delay in relapse would be in the order of several years. The relative merits of pure antiestrogens or tamoxifen analogs (such as raloxifene or idoxifene) as opposed to aromatase inhibitors in terms of a beneficial effect on bone mineral density, endometrium and lipid profile may then become important in the choice of an effective long-term adjuvant endocrine agent.

Conclusions

There are likely to be several different mechanisms for tamoxifen resistance in human breast cancer *in vivo* (Table 1). The ER remains crucial in determining a tumors' primary response to tamoxifen and lack of expression of a functionally active ER appears to be the major factor responsible for *de novo* resistance. In contrast acquired resistance in many tumors is often associated with maintained ER expression. Several potential sites for alteration in the ER-mediated signal transduction pathway have been examined *in vivo* and recent experimental data have postulated further downstream molecular processes which may be involved. The elucidation of all these pathways may provide a better understanding of the complex molecular pharmacology of tamoxifen and may provide a unifying hypothesis for the tissue-specific effects of the drug as well as the growth-stimulatory properties which may account for acquired resistance. On the basis of these studies novel molecular targets in tamoxifen-resistant tumors may be identified. For the immediate future, the emergence of novel endocrine therapies provides a challenge to see whether clinical therapeutic strategies can be devised to overcome tamoxifen resistance in breast cancer.

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