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Tamoxifen resistance in breast cancer

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Contents

Tamoxifen (TAM) resistance is the underlying cause of treatment failure in many breast cancer patients receiving TAM. The mechanism(s) involved in TAM resistance are poorly understood. A variety of mechanisms have been proposed but only limited evidence exists to substantiate them. Studies have now shown that in many patients TAM resistance is not related to the down regulation or loss of estrogen receptors (ER). Variant ER have been identified, but their significance clinically remains to be proven. Since breast cancer cells secrete several estrogen-regulated growth factors and growth inhibitors that may have autocrine or paracrine activity, altered growth factor production is another possible mechanism for TAM resistance. Tissue-specific transcription activating factors that may alter how the signal induced by TAM binding to the receptor is interpreted by the cell also require further investigation. An increase in antiestrogen binding sites (AEBS), which could effectively partition TAM and reduce its concentration at the ER has also been proposed as a potential mechanism. Pharmacologic mechanisms, such as a shift in metabolism toward the accumulation of estrogenic metabolites, are supported by recent data demonstrating metabolite E and bisphenol in tumors from TAM-resistant patients. Furthermore, a decrease in tumor TAM accumulation and an altered metabolite profile have been reported in TAM-resistant breast tumors

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grown in nude mice. These and other studies suggest that TAM resistance may be multifactorial in nature, but definitive identification of mechanisms that are operative in clinical TAM resistance requires further study.

I. Introduction

Tamoxifen (TAM) is a nonsteroidal antiestrogen that was originally synthesized in 1966 as an antifertility drug [1]. However, in the 1970s TAM was noted to have activity in the treatment of metastatic breast cancer, and clinical trials began in the United States in 1974. In 1978 TAM was primarily used to treat postmenopausal women with estrogen receptor positive, metastatic breast cancer. However, its clinical role has expanded to include all stages of the disease (Stage I and II) and both pre- and postmenopausal patients [2]. Response rates and duration of response in studies comparing TAM and oopherectomy in premenopausal patients are similar. Response rates to TAM increase with higher tumor ER levels. Overall, TAM prolongs both the disease-free and overall survival of women following primary surgery [3], and it induces tumor regression in about half of women with advanced estrogen receptor positive, metastatic breast cancer [4]. TAM has demonstrated efficacy in the prevention of contralateral breast cancer and it is also currently being evaluated for use as a chemopreventative agent in healthy women at high risk of breast cancer.

Although approximately 50% of estrogen receptor-positive (ER+) tumors will respond to TAM, only 60–75% of patients with metastatic breast cancer have estrogen receptor-positive tumors. Therefore only 35% of metastatic breast cancer patients actually benefit from TAM therapy [5]. In addition, all patients who initially respond to therapy will eventually develop acquired TAM resistance following prolonged administration.

The development of acquired TAM resistance, where cell populations initially sensitive to TAM become insensitive, differs from innate resistance where cell populations are insensitive to TAM from the onset of

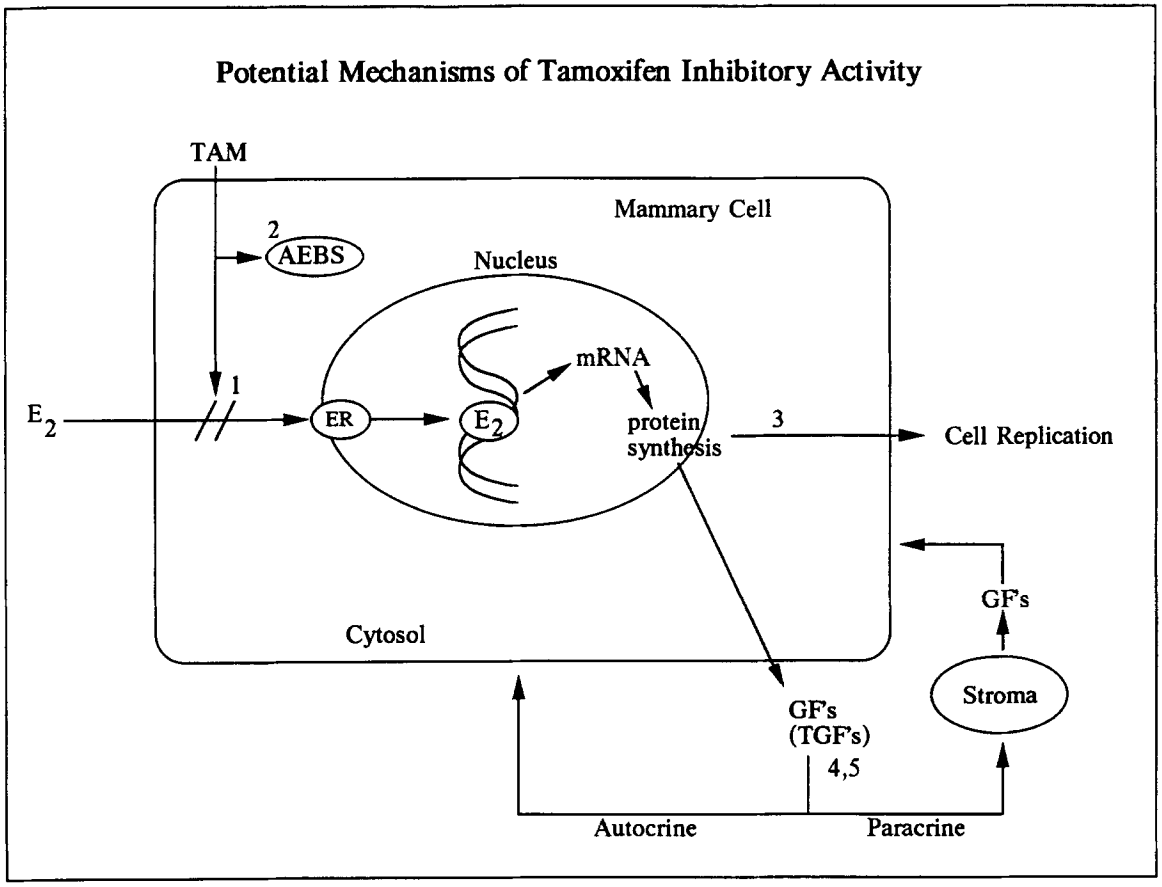

Fig. 1. Shows the mechanism of estrogen (E2) binding to the estrogen receptor (ER) and the growth inhibitory effects of tamoxifen (TAM). TAM competitively blocks the binding of E2 to the ER (1), it also binds to the antiestrogen binding sites (AEBS) (2). TAM blocks cells in G0/G1 (3) inhibiting cell replication. TAM may also decrease concentrations of TGF-α, a growth factor that is stimulatory (4) and may increase levels of TGF-β, an inhibitory growth factor (5).

drug exposure. Unfortunately, the cellular and molecular mechanisms underlying the development of acquired resistance to antiestrogens remains unclear. However, a variety of potential mechanisms have been suggested, and several reports concerning potential mechanisms for acquired TAM resistance have recently been published.

II. Mechanism of antiestrogen action

The mechanism(s) by which TAM inhibits tumor cell growth are believed to be mediated is primarily through interaction with ER (Fig. 1). Competitive antagonism of estrogen at the ER by TAM slows the growth of estrogen-dependent cancer cells by blocking them in the G0/G1 phase of the cell cycle [6]. Binding of TAM to the receptor is believed to form a complex that, when bound to estrogen-response elements, fails to trigger transcription of target genes. The resulting blockade is believed to be predominantly cytostatic in nature and may be reversed by the addition of estradiol. Whether TAM induces apoptosis or cell death is not yet clear.

The antiestrogenic activity of TAM has also been evaluated in several species, and the biological effects of the drug appear to be dependent both on the species studied and the target tissue examined. In rats and humans, TAM has similar biological activity. In both species TAM has partial estrogen agonist effects on uterine tissues, but it is primarily considered an estrogen antagonist [7]. TAM's weak estrogenic like effects have also been noted in postmenopausal patients in whom estrogenic effects were noted on gonadotrophin levels, plasma proteins and vaginal epithelium [7–9]. Whether the difference in antiestrogenic action is related to species specific or tissue specific metabolism of TAM, or to the presence of specific transcription factors that alter signal interpretation by the cell following interaction of the antiestrogen with the estrogen receptor is unknown. However, many other factors may also play a role in the cellular response to TAM.

Several studies have now shown that cellular inhibition by TAM may involve a complex series of events. Modulation of breast cancer cell growth by the differential stimulation or inhibition of growth factor production from cells may also be involved in antiestrogen action. Recent evidence now suggests that estrogens may stimulate cell growth in part by inducing cells to synthesize growth factors and/or receptors. TAM on the other hand may act by inhibiting the estrogen-induced production of growth factors, while at the same time stimulating the production of growth inhibitory factors.

At least one pathway may involve the stimulation of transforming growth factor-beta (TGF-β) production by TAM. TGF-β has both growth inhibitory and stimulatory effects. In stromal cells such as fibroblasts or endothelial cells, it stimulates cell growth. However, in most types of epithelial tumor cells, including breast tumors, it acts as a growth inhibitor [10]. Although the exact mechanism of TGF-β growth inhibitory effects are poorly understood, it does appear to inhibit tumor cell growth independent of the ER. A number of other growth factors are produced by breast cancer cells, and their expression is modified by estrogens and antiestrogens. These include TGF-alpha, IGF-II, PDGF, and members of the EGF family [11]. However, the exact role that each of these growth factors plays in the induction of cell growth by estrogen and the inhibition of cell growth by TAM remains to be elucidated.

TAM has also been noted to bind to sites that are independent of the ER. These high affinity binding sites (K_d = 1 nM) are referred to as antiestrogen binding sites (AEBS) [12]. AEBS have been identified in several tissues with the highest concentrations noted in liver, uterus, ovaries, brain, and kidneys [13]. AEBS appear to be distinct from the ER and are only observed following prior treatment with estradiol [12].

The affinity of antiestrogens for AEBS does not closely correlate with the biological potency of antiestrogens, suggesting that AEBS do not directly mediate antiestrogen action [14,15]. Many studies have attempted to correlate binding of AEBS to other cellular events related to antiestrogen actions including protein kinase C inhibition [16], calmodulin inhibition [17] and interactions with a variety of receptors, including histamine [18], dopamine [19] and muscarinic receptors [20]. Indeed much interest has been placed on the study of AEBS over the years; however, their true function and role in the antitumor efficacy of TAM remains to be established.

III. Potential mechanisms of acquired tamoxifen resistance

A variety of mechanisms has been implicated in the development of acquired resistance to TAM. However, little definitive data are available to support many of the proposed mechanisms. At one time it was assumed that drugs were responsible for inducing some biochemical modification in cells that resulted in acquired resistance to that drug. However, for many types of drug resistance the drug does not play a direct role in the development of resistance, but instead provides a strong selective pressure in favor of drug-resistant subclones. Drug-resistant subclones resulting from spontaneous mutations differ genetically from the original population. A

deletion or modification of a specific enzyme, or alteration in some other cellular property in the genetically altered cell population may be responsible for the altered sensitivity of cells to the drug.

Recent evidence derived from in vitro and in vivo studies suggests that TAM can stimulate cells to grow following prolonged exposure [21–23]. Whether TAM is selecting a subclone of TAM-stimulated cells, whether the cells are altering TAM in such a way as to generate a stimulatory signal, or whether TAM is capable of inducing a genetic mutation that results in altered sensitivity to the drug remains to be established. One study has now shown that TAM can produce DNA adducts in the liver of rats suggesting that it may have genotoxic activity that could theoretically lead to mutations [24].

Although the mechanisms underlying TAM resistance remain vague, a variety of recent studies suggest that multiple mechanisms may contribute to TAM resistance. Studies examining (A) altered level of ERs, (B) a decrease in ER affinity, (C) enhancement of cellular mechanisms for bypassing TAM cytotoxicity, (D) decreased cellular TAM concentration, (E) increased concentration of antagonizing metabolites and (F) a variety of other pathways, have contributed to our understanding of the possible mechanisms underlying TAM resistance.

III-A. Altered levels of estrogen receptor

III-A.1. Clinical clues

ER expression is regulated to meet the demands of the cell. In the presence of high concentrations of estradiol, down regulation of receptors is believed to occur. The absence or loss of estrogen receptors could explain the development of hormonal independence or TAM resistance, particularly since ER-negative tumors rarely respond to TAM. However, clinical studies suggest that resistance to TAM is not always caused by selection of a hormone independent and/or ER-negative clone of tumor cells. Sequential biopsy studies have shown that apparent loss of ERs is common when the second biopsy is performed while the patient is taking TAM or within two months of stopping TAM presumably, due to receptor occupancy by the drug causing a false-negative ligand binding assay [25]. When the second biopsy is performed after two months, tumors frequently remain ER-positive, suggesting that there is no selection of a truly ER-negative clone. In a recent study of tumors from patients with TAM-resistance, an immunohistochemical technique was used to detect both bound and free receptors, ER was found in seven out of 13 tumors examined [26]. Maintenance of ER and/or PgR levels and responses to secondary hormonal therapies are not uncommon in patients with acquired TAM resistance [4,25]. Furthermore, in vitro studies suggest that following the selection of antiestrogen-resistance cells, many resistant cell lines may remain sensitive to estrogens [27,28].

Thus, ER loss may play a role in acquired TAM resistance in some patients, but it cannot account for the resistance noted in the majority of patients. In addition, clinical evidence suggests that patients that initially respond to TAM, but who later develop resistance, frequently respond to secondary hormonal treatment, suggesting that the development of resistance to antiestrogens does not confer resistance to other hormonal agents [4].

III-B. Altered estrogen receptor

Protein structure modifications leading to altered affinity of the ER for TAM is a plausible resistance mechanism. Site-specific mutations, including nonsense or frameshift mutations in the structural gene coding for the ER may potentially result in various types of functionally abnormal receptors. These mutations may render the ER entirely nonfunctional; thus, the tumor would appear clinically as if it were ER-negative. Alternatively, if mutations result in amino acid substitutions in important domains of the receptor, then the result may be the generation of ER species which are functionally active, but which exhibit altered specificities for estrogens and antiestrogens.

III-B.1. ER variants

Much is known about the structure and function of the ER [29]. The ER contains discrete domains involved in hormone binding, DNA binding, and subsequent activation of estrogen-responsive genes. Human ERs have now been shown to contain five distinct functional domains A/B, C, D, E and F [30]. Although there is some overlap between domains, regions E and D appear to primarily involve the hormone-binding and dimerization domains [30,31]. Region C is the DNA binding domain, and the A/B and E regions contain the two transcription activating functions.

The presence of these discrete functional domains has led investigators to examine alterations in TAM-resistant, or hormone-independent model systems. Many earlier studies failed to show differences in the ER in in vitro systems. For example, Mullick and Chambon [32] used two independently isolated TAM-resistant, ER-positive breast cancer cell lines, LY2 and T47D, to demonstrate that the ER was still functional in these

cells in spite of their hormone-insensitive growth. The ER was shown to be wild-type using RNase protection assays and by its ability to stimulate estrogen-responsive reporter constructs in these cells. However, neither of these assays rule out the possibility that mutated ER species may be present along with wild-type ER, and that these mutated forms may contribute to the tamoxifen-resistant phenotype. Direct sequence analysis of the ER from these cells may be required to definitively answer this question.

In fact, Graham et al. [33] have examined the presence of mutated ERs by cloning and sequence analysis in T47D cells that have been maintained in their laboratory. Several different ER mutations were detected in complementary DNAs prepared from these cells, including frameshift mutations within the DNA and the hormone binding domains of the receptor. If expressed, these mutated ER species could be defective in activity, and could contribute to the hormone-independent phenotype of this T47D subline. Of importance from this study is that highly sophisticated technologies were required to identify ER mutations present in cells which were heterogenous for ER expression.

Raam et al. [34] have utilized immunohistochemical procedures to demonstrate the presence of ERs deficient in nuclear binding in ER-positive tumors. Interestingly, patients with either constitutive nuclear binding, or those with ER which could not bind nuclei, were refractory to hormone therapy. This encouraging result, that tumors may contain defective ERs, has been recently substantiated by studies using larger series of human breast tumor specimens [35,36]. Even though endocrine response data was not available on these later studies, both studies suggest that truncated forms of the ER, which fail to bind DNA in gel-retardation assays, may be present in tumors. Of note, is that DNA binding-deficient ER was most prevalent in tumors with minimal PgR expression, agreeing with the commonly-held doctrine that PgR expression closely correlates with an intact ER response pathway. It will be interesting to apply the gel-retardation methodology to tumors with clinical response data to determine whether ERs defective in DNA binding may contribute to TAM failure.

Murphy et al. [37] have identified abnormal sized ER mRNAs by Northern hybridization, and they have recently cloned these altered ERs from human breast tumors. Three different ER mRNAs have been identified, all of which diverge from the known ER sequence at exon/intron borders. At the point of divergence, non-ER sequences have been inserted. These insertions are either unknown or are homologous to long interspersed repetitive LINE-1 sequences. These three altered ERs are all missing the hormone binding domain of the receptor in addition to containing unique non-ER segments. One of the mutated ERs, designated clone 4, is widely expressed in breast tumor samples (Dozlaw, unpublished results). Although clone 4 ER was devoid of transcriptional activity in in vitro assays, its presence may be functionally significant in tumors. Experiments are currently underway to examine its distribution in breast tumors where clinical information is available (Murphy and Fuqua, unpublished observations).

The progression of steroid sensitive cells to steroid insensitivity was evaluated by Darbe and King [38]. In their study they demonstrate that clones of steroid-responsive cells can give rise to a population of unresponsive cells in a series of phenotypic changes which are brought about solely by long-term withdrawl of the hormone. In a further study they report that transfection of a steroid inducible gene into unresponsive cells (S115) results in that gene being fully inducible by steroids. Therefore, the machinery for steroid responsiveness, including receptors, appears to be intact. They suggest that the process appears to be independent of the loss of steroid receptor function [39]. This was further substantiated by Clarke et al. who examined the progression of human breast cancer cells from hormone-dependent to hormone-independent growth in vitro and in vivo. They report that an ovarian-independent, but hormone-responsive phenotype may occur early in the natural progression to hormone-independence, but that altered hormone receptor expression may be a late event in the acquisition of this phenotype [40].

ER variants have also been isolated from a variety of ER-positive, and supposedly ER-negative breast tumors, using sensitive RNA-directed polymerase chain reaction (PCR) methodologies [41,42]. Tumors which are ER-negative, but PgR-positive often express high levels of a variant ER lacking exon 5 of the hormone binding domain of the receptor. This deletion results in the production of a variant ER truncated within the hormone binding domain, and as such, is unable to bind estrogen. However, the receptor appears to bind DNA and is constitutive for activation of estrogen-responsive genes. When the exon 5 ER variant is coexpressed with wild-type receptor in MCF-7 cells, TAM-resistant growth is conferred to these cells. Thus, overexpression of the variant, even in the presence of wild-type receptor, may contribute to TAM resistance. Furthermore, ER-positive tumors which express wild-type ER often coexpress the exon 5 ER deletion variant. Tumors with this variant may escape the normal growth dependence of estrogens, and subclones may be selected for under

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