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The estrogen receptor from a tamoxifen stimulated MCF-7 tumor variant contains a point mutation in the ligand binding domain

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Key words: breast cancer, tamoxifen, drug resistance, estrogen receptor mutant

Abstract

The nonsteroidal antiestrogen tamoxifen (TAM) is the most commonly used endocrine treatment for all stages of breast cancer in both pre- and postmenopausal women. However, the development of resistance to the drug is common, as most patients treated with TAM eventually experience a recurrence of tumor growth. One of the potential mechanisms of treatment failure is the acquisition by the tumor of the ability to respond to TAM as a stimulatory rather than inhibitory ligand. We (Gottardis and Jordan, Cancer Res 48: 5183–5187, 1988; Wolf *et al.*, J Natl Cancer Inst 85: 806–812, 1993) and others (Osborne *et al.*, Eur J Cancer Clin Oncol 23: 1189–1196, 1987; Osborne *et al.*, J Natl Cancer Inst 83: 1477–1482, 1991) have extensively described the reproducible development of TAM stimulated growth in a laboratory model system using MCF-7 human breast cancer cells grown as solid tumors in athymic mice. In this paper we report on the isolation of an estrogen receptor (ER) from a TAM stimulated tumor (MCF-7/MT2) which contains a point mutation that causes a tyrosine for aspartate substitution at amino acid 351 in the ligand binding domain. The mutant appears to the major form of ER expressed by this tumor. We also report that only wild type ER was detected in three other TAM stimulated MCF-7 tumor variants, suggesting that multiple mechanisms are possible for the development of TAM stimulated growth. The implications of these findings are discussed.

Introduction

MCF-7 tumors grown in athymic mice chronically treated with tamoxifen (TAM) reproducibly develop a TAM stimulated phenotype [1–4]. Previous reports [3, 5, 6] have suggested that the development of TAM stimulated growth was associated with a decrease in the intracellular level of TAM and an increase in the conversion of TAM and TAM metabolites to compounds with reduced antiestrogenic and/or heightened estrogenic activity. We addressed this hypothesis in a recent paper [4]. We treated tumor bearing mice with a TAM analog (fixed-ring TAM) that could not be converted to weakly antiestrogenic or potent estrogenic compounds, and we used HPLC to measure the intratumoral concentrations of TAM and its metabolites. We found that TAM and fixed-ring TAM were equally capable of developing and maintaining TAM stimulated growth after chronic exposure, and that levels of TAM and metabolites did not differ between TAM inhibited and TAM stimulated tumors. We concluded that TAM stimulated growth of the tumor models maintained in our laboratory

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was not associated with alterations in TAM transport or metabolism. Therefore we undertook the experiments described in this and the accompanying report [7] to investigate what alterations in tumor characteristics might be associated with the development of TAM stimulated growth.

Work in many laboratories, including our own, has shown that alterations in the sequence of a steroid receptor can dramatically alter its activity in response to a ligand. Vegeto *et al.* [8] have shown that truncation of the extreme C-terminus of the progesterone receptor (PR) causes a loss of response to progestins, but confers full agonist activity on the antiprogestational agent RU 486. Also, the androgen receptor in the prostate carcinoma cell line LnCAP contains a point mutation which causes these cells to respond to antiandrogens, estrogens, and progestins as androgen receptor agonists [9–13].

Similarly, a point mutation in the ER can markedly alter the pharmacology of antiestrogenic ligands [14–16]. Jiang and Jordan [14] stably transfected the estrogen receptor (ER) negative breast cancer cell line MDA-MB-231 with either wild type (HEGO) or mutant (HEO) ER. The mutant ER contains a valine for glycine substitution at position 400 in the hormone binding domain. E_2 retards the growth rate of cells containing either the wild type or the mutant receptor, and the pure antiestrogen ICI 164,384 returns growth to control levels [14]. In cells transfected with the wild type ER the potent antiestrogens 4-OHT and RU 39,411 also blocked growth inhibition by E_2, returning growth almost to control levels. However, in cells transfected with the mutant ER, E_2, 4-OHT, and RU 39,411 inhibited growth [15, 16]. Thus, in cells containing the mutant ER, both E_2 and the antiestrogens 4-OHT and RU 39,411 act as ER agonists, but the pure antiestrogen ICI 164,384 does not.

The MCF-7 TAM tumor developed by Gottardis and Jordan [2] has similarities to MDA cells transfected with the mutant HEO ER, because both TAM and E_2 acted as agonists for growth [2], whereas the pure antiestrogen ICI 164,384 inhibited TAM stimulated tumor growth [17]. In the accompanying paper [7] we report the generation of three new TAM stimulated MCF-7 tumor variants, two which have phenotypes similar to early passages of the MCF-7 TAM tumor (i.e., they are growth stimulated by both E_2 and TAM) and one which has a phenotype similar to late passages of the MCF-7 TAM tumor, in that it grows in TAM treated athymic mice, but not in E_2 or placebo treated animals [18, 19]. We performed the experiments described in this report to determine if the development of TAM stimulated growth by MCF-7 tumor derivatives might be associated with the expression of a mutated ER.

To determine if tumors expressed a mutant ER, we performed polymerase chain reaction (PCR) single strand conformational polymorphism (SSCP) analysis of ER cDNAs prepared from tumor RNA. SSCP analysis is based on the principal that the mobility of a single-stranded DNA molecule on a non denaturing gel is determined by both its length and its secondary structure [20–22]. The technique is sensitive enough that a single base difference between two otherwise identical single-stranded DNA molecules (less than about 600 bases long) is sufficient to give them different mobilities on a non denaturing polyacrylamide gel. Using SSCP analysis, we found that three TAM stimulated tumors (MCF-7 TAM, MCF-7/MF1 and MCF-7/MT3) express wild-type ER, but another TAM stimulated tumor (MCF-7/MT2) expresses an ER with a point mutation in its ligand binding domain.

Materials and methods

Athymic mice and tumor transplants

MCF-7 and MCF-7 TAM breast tumors were maintained as serially passaged solid tumors in ovariectomized athymic nude mice (Harlan Sprague Dawley, Madison, WI) treated with either estradiol (E_2) or TAM capsules. Serial passaging procedures and capsule preparation have recently been described in detail [23]. Tumors used in the experiments described in this paper were derived from the studies of TAM stimulated tumor growth described in the accompanying paper [7].

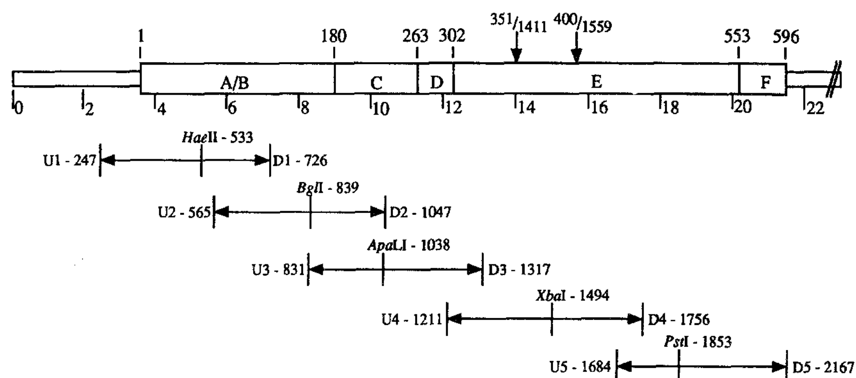

Fig. 1. PCR/SSCP strategy. The top portion of the figure contains a schematic diagram of the ER cDNA. Nucleotide positions in hundreds of base-pairs are indicated on the bottom, and codon positions are indicated along the top. Letters in the larger rectangles indicate domains of the receptor: A/B – N-terminal region, C – DNA binding domain, D – hinge region, E – ligand binding domain, F – C-terminal region. The positions of codons 351 and 400, and the bases changed in each of those codons, are indicated above the diagram by arrows. The bottom portion of the diagram shows the 5 segments of the ER which were PCR amplified for SSCP analysis. The primers (see Table 1) and nucleotide termini for each segment are indicated at the ends of the arrowheads in the Figure. The positions at which each restriction enzyme used for SSCP cut the coding strand are indicated above the relevant segment.

RNA isolation

Total RNA was prepared from tumors by the method of Chomczynski and Sacchi [24]. Tumors were pulverized in liquid nitrogen and the resulting powder was homogenized in guanidinium isothiocyanate buffer in a Dounce pestle homogenizer. The resulting homogenate was extracted with acidified phenol and total RNA was recovered by isopropanol precipitation from the aqueous phase (see [7] for additional details).

Polymerase chain reaction amplification and single strand conformational polymorphism analysis

SSCP analysis was performed using the method of Orita *et al.* [20, 21], with modifications of Iwahana *et al.* [22]. Tumor total RNA (5 mg) was reverse transcribed in a 50 μl reaction containing 50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 0.5 mM each dATP, dCTP, dGTP, dTTP, 10 mM dithiothreitol, 3 μM oligo dT_{16}, 100 U placental RNase inhibitor (Promega, Madison, WI), and 200 U Moloney murine leukemia virus reverse transcriptase (United States Biochemical, Cleveland, OH). PCR amplification and ^{32}P labeling were carried out in a 50 μl reaction containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dATP, dCTP, dGTP, dTTP, 10 μCi ea. ^{32}P-α-dATP and dCTP (3000 Ci/mmol, Dupont NEN, Hoffman Estates, IL), 0.15 μM each primer, and 2.5 U *Taq* DNA polymerase (Boerhinger Mannheim, Indianapolis, IN). Five μl of the reverse transcription mixture was used as a template in the amplification/labeling re-

Table 1. Primers used for PCR/SSCP amplification of ER cDNA

Primer name[1]	Position[2]	Sequence[3]
U1	247–266	ATG CGC TGC GTC GCC TCT AA
D1	726–707	CTG CAG GAA AGG CGA CAG CT
U2	565–584	AAC GCG CAG GTC TAC GGT CA
D2	1047–1028	AAT GGT GCA CTG GTT GGT GG
U3	831–850	ACG CCA GGG TGG CAG AGA AA
D3	1317–1298	CAA GGC ACT GAC CAT CTG GT
U4	1211–1230	GAG ACA TGA GAG CTG CCA AC
D4	1756–1737	GGG TGC TGG ACA GAA ATG TG
U5	1684–1703	GGA GAG GAG TTT GTG TGC CT
D5	2167–2148	TGT GGG AGC CAG GGA GCT CT

[1] Primer names indicate which region of the ER (1–5, see Fig. 1) they were used to amplify. U and D indicate whether the primer corresponds to the upstream or downstream end of a given fragment, respectively. U primers are identical to the coding strand sequence, D primers are complementary.

[2] Numerical base positions correspond to those given in the GenBank sequence for the human ER. The nucleotide corresponding to the 5′ end of the primer is listed first.

[3] All sequences are listed 5′ to 3′.

action. Control amplifications were carried out on 50 pg double stranded DNA coding for either a wild type ER (HEGO), or a mutant ER (HEO) which contains a single G to T point mutation at nucleotide 1559 [25, 26]. Both ER cDNAs were the generous gift of Professor Pierre Chambon, Strasbourg, France. Amplifications were carried out for 40 cycles of 94° C for 1 min, 60° C for 1 min, and 72° C for 2 min. A final 7 min incubation at 72° C was carried out to insure complete extension.

The ER cDNA was amplified and ^{32}P labeled in 5 overlapping section using the strategy diagrammed in Fig. 1. Primer sequences and positions at which they anneal are indicated in Table 1. ER cDNA from the reverse transcription reaction was amplified using one of the primer pairs shown, and an aliquot of the resulting ^{32}P labeled DNA was digested with the restriction endonuclease indicated in Fig. 1 for a given primer pair. Following restriction digestion, an aliquot of cut and uncut DNA was diluted 1:4 with 0.1% SDS, 10 mM EDTA. Half of each diluted sample was mixed 1:1 with non-denaturing loading buffer (50% glycerol, 0.05% bromophenol blue, 0.05% xylene cyanol), and the remaining half was mixed 1:1 with denaturing loading buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol). Non-denatured samples were loaded without further manipulation onto 5% acrylamide gels containing 0.5×TBE (45 mM Tris-HCl, 45 mM boric acid, 2 mM EDTA, pH 8.3) and 5% glycerol. Denatured samples were heated to 80° C for 3–5 min and loaded immediately. Gels were run at room temperature with 14–16 watts constant power for 4 to 6 h with a cooling fan. Gels were dried in a vacuum gel dryer and exposed to film (Fuji RX, Fuji Photo Film Co. LTD., Tokyo, Japan) overnight at –70° C.

Cloning and dideoxy sequencing of MCF-7/MT2 ER fragment

The ER fragment 4 (see Fig. 1) from MCF-7/MT2 tumors was PCR amplified using the reaction conditions described above, except that no ^{32}P labeled nucleotides were included in the reaction mixture. The PCR product was treated with the Klenow fragment of DNA polymerase I (Promega, Madison, WI) to remove the 3′ overhanging A residues left by *Taq* DNA polymerase, and then digested with *Pst*I, which cleaves at nucleotide 1681. The resulting fragment was ligated into the plasmid vector pBSK+, which had been digested with *Sma*I and *Pst*I to generate a cloning site complementary to the ER fragment. The resulting plasmid was transformed into competent *E. coli* (DH5α strain) by the method of Chung and Miller [27], and plated on LB plates containing 50 μg/ml ampicillin and 20 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal).

Colonies were inoculated into LB containing 50 μg/ml ampicillin and grown overnight. Plasmid was isolated from these cultures using a Magic Minipreps DNA purification kit (Promega, Madison, WI). The isolated plasmids were digested with *Bam*HI and *Eco*RI to release the insert, and run on a 1% agarose gel containing 0.5 μg/ml ethidium bromide to check insert size. Plasmids containing appropriate size inserts were sequenced by the dideoxy method using the Sequenase version 2.0 DNA sequencing kit (United States Biochemical, Cleveland, OH). ^{35}S-α-dATP (1000–1500 Ci/mmol, Dupont NEN, Hoffman Estates, IL) was used as a label. Sequencing reaction products were run on 6% acrylamide gels containing 7 M urea, and buffered with extended range TBE: 130 mM Tris-HCl, 45 mM boric acid, 2.5 mM EDTA, pH 9.0 (Biorad, Richmond, CA). Gels were fixed in a bath of 10% acetic acid, 10% methanol and dried in a vacuum gel drier. Gels were exposed to film (Kodak XAR, Eastman Kodak, Rochester, NY) overnight at –70° C.

Results

SSCP analysis of tumor ERS

PCR amplification and ^{32}P labeling of ERs from MCF-7 derived tumors was carried out using the primer pairs shown in Fig. 1. ER cDNAs from the following tumors were analyzed: a wild type MCF-7 tumor at serial passage 6, the TAM stimulated tumor variant MCF-7 TAM developed by Gottardis

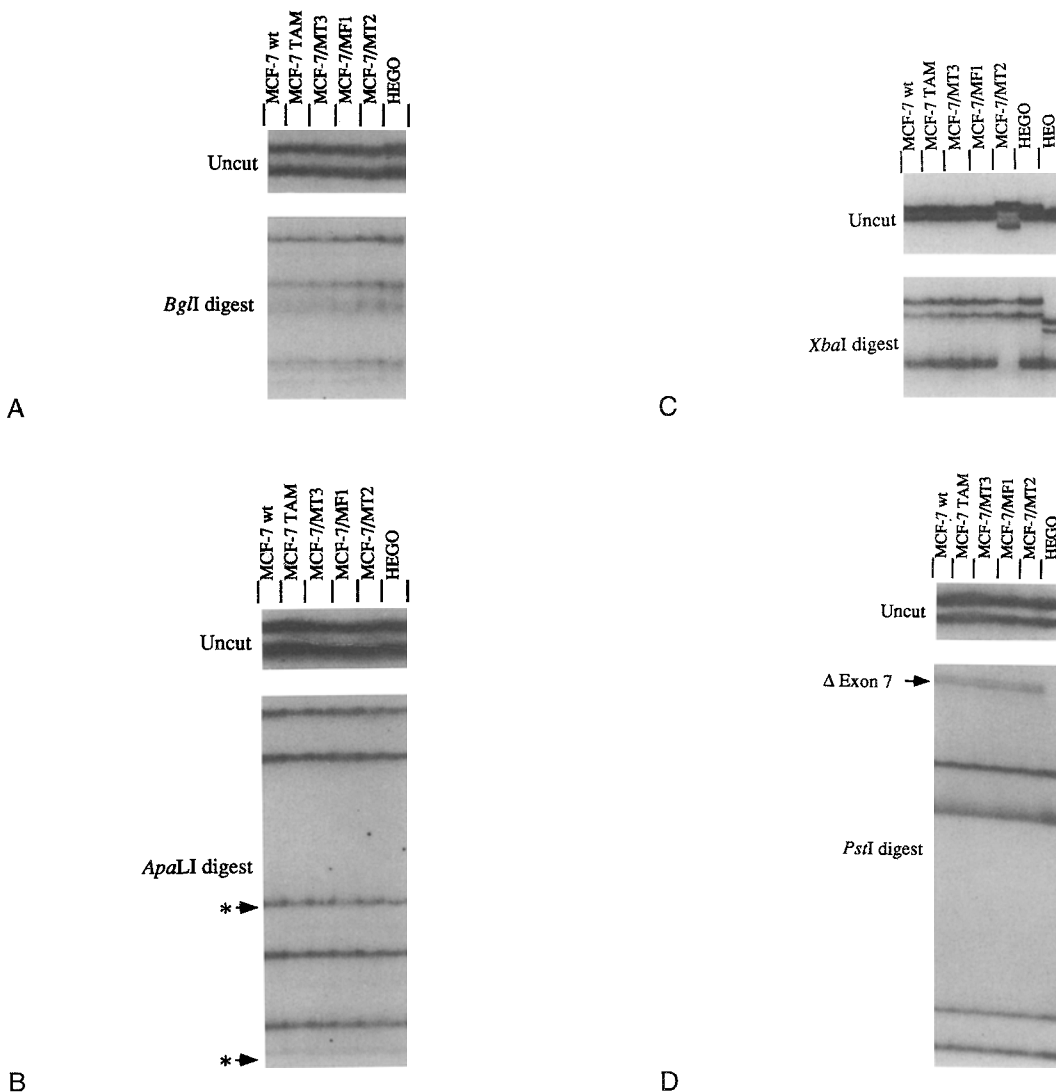

Fig. 2. SSCP analysis of ER from 5 MCF-7 derived tumors. Ranges of SSCP analysis are as shown in Fig. 1. A) Region 2, from base pair 565 to base pair 1047. B) Region 3, from base pair 831 to base pair 1317. C) Region 4, from base pair 1211 to base pair 1756. D) Region 5, from base pair 1684 to base pair 2167. In all cases, the first 5 lanes contain PCR/SSCP products from the indicated tumors. The lane labeled HEGO contains DNA amplified from the wild-type ER cDNA HEGO. In C, the lane labeled HEO contains DNA amplified from a cDNA encoding the mutant ER HEO. In all cases, the upper panel shows the migration of the intact single stranded DNAs. The lower panel contains the single stranded products resulting from restriction digestion by the indicated restriction endonuclease digestion. In B, the asterisks indicate double stranded bands due to incomplete sample denaturation. In D the bands marked Δ Exon 7 are produced by amplification of an exon 7 deletion variant of the ER [28], which is ubiquitous in MCF-7 derivatives.

and Jordan [2], at serial passage 22, and three new TAM stimulated MCF-7 derived tumors, MCF-7/MT2, MCF-7/MT3, and MCF-7/MF1, which have recently been described [7]. All tumors used in this experiment were taken from animals initially treated with the 'normal' stimulatory ligand for that tumor (E_2 for MCF-7 and TAM for MCF-7 TAM, MCF-7/MT2, MCF-7/MT3, and MCF-7/MF1), and then switched to placebo for 3 to 4 weeks prior to sacrifice. Tumors treated in this fashion were used because they express the highest levels of ER RNA [7]. The cloned ER cDNA for the wild type receptor

was used as a control. In addition, an ER cDNA (HEO) containing a G to T point mutation at nucleotide 1559 was run in the experiment using primer set 4 (see Fig. 1) in order to test the sensitivity of the SSCP technique.

In all cases except one, tumor samples produced bands that comigrated exactly with those produced by amplification of the wild type ER cDNA (Fig. 2). The only exception occurred in the analysis which used primer set 4, which amplifies a region encoding the upstream half of the ligand binding domain (Fig. 1). Figure 2C shows the results of this experiment. The bands in the upper panel are the denatured strands of the full length 546 bp PCR products. The lower panel shows the denatured products resulting from digestion of the full length product with *Xba*I, which cleaves after nucleotide 1494 on the coding strand and after nucleotide 1498 on the complementary strand, producing 4 single stranded bands of 288, 284, 262 and 258 bases in length. Most of the tumor samples produced bands which migrated identically with that produced by the wild type ER cDNA. However, the products of reactions run on the MCF-7/MT2 sample and on the mutant ER cDNA HEO, have a migration pattern different from the wild type ER. Further, Fig. 2C shows that the MCF-7/MT2 and HEO products differ from each other as well as from wild type, indicating the mutation in the MCF-7/MT2 ER is not a G to T transversion at nucleotide 1559. The location of the MCF-7/MT2 mutation can be narrowed down based on the results shown in the lower panel of Fig. 2C. The upper doublet is constant in the first 6 lanes, but has increased mobility in the HEO lane, indicating that it corresponds to the region from the *Xba*I site at nucleotide 1494 to the end of the fragment at nucleotide 1756 (see Fig. 1). In contrast, the lower bands have changed only in the MCF-7/MT2 lane, which indicates that the mutation in the MCF-7/MT2 ER lies between nucleotide 1211 and the *Xba*I site at nucleotide 1494 (see Fig. 1). It is not clear why the DNA fragments produced from the MCF-7/MT2 ER in the lower panel of Fig. 2C have become faint and diffuse rather than simply altering in mobility, but it is nevertheless obvious that their migration differs from the wild type ER sequence.

At the top of the *Pst*I digest panel in Fig. 2D, a pair of bands is visible in all 5 MCF-7 derived samples, but is absent in the sample prepared from HEGO cDNA. These bands are the result of amplification of an ER sequence lacking exon 7 [28]. This variant is present in all MCF-7 derived cell lines and tumors that we have tested (Wolf, Pink, and Jordan, unpublished observations). Note that Fig. 2 only shows SSCP analyses of ER regions 2–5 (as indicated in Fig. 1). Data from region 1 is not included as all bands ran as diffuse smears similar to that observed in the XbaI cut MCF-7/MT2 lane in Fig. 2C. No differences in migration were observed, but the data is such that firm conclusions regarding mutations occuring between base pair 247 and 565 cannot be made. This region includes 204 coding bases between base pair 361 and 565.

Cloning and sequencing of the MCF-7/MT2 ER fragment

In order to determine the nature of the mutation in the MCF-7/MT2 ER, we PCR amplified the ER region flanked by primer set 4 and cloned the result-

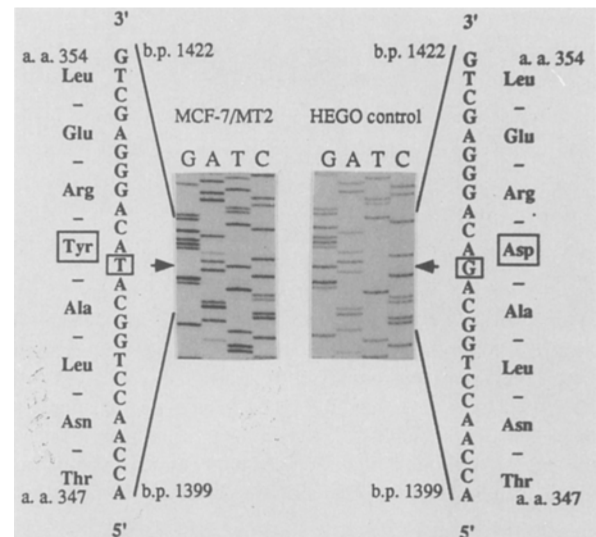

Fig. 3. Sequence analysis of MCF-7/MT2 ER. The left panel shows the sequence of one of the three ER segment 4 clones isolated from the MCF-7/MT2 tumor. The panel on the left is a sequence of the same region of the wild-type ER cDNA HEGO, for comparison. The nucleotide and amino acid affected by the change are shown in boxes on each side of the Figure.

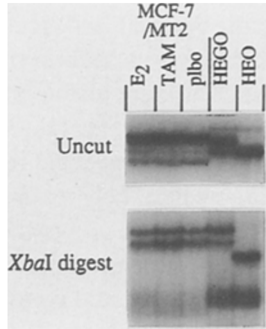

Fig. 4. Confirmation of ER mutation in multiple MCF-7/MT2 tumors. PCR SSCP was performed as described for Fig. 2. The first 3 lanes were derived from three MCF-7/MT2 tumors taken from three different mice. Lane 1 is from an E2 treated animal, lane 2 from a TAM treated animal, and lane 3 from an animal implanted with a placebo capsule. The upper and lower panels are as described in the legend to Fig. 2.

ing fragment into the multicloning site of the plasmid pBSK+ using the strategy outlined in Materials and methods. The resulting plasmid was transformed into *E. coli* and white colonies were selected after plating on X-gal containing plates. Plasmid was isolated from minipreps done on several white colonies, and the three plasmids that contained inserts of the appropriate size were sequenced using primers complementary to the T3 and T7 promoters which flank the multicloning site of plasmid pBSK+. All three plasmids contained identical inserts which matched the published ER sequence, except that they contained a T instead of a G at position 1411, as shown in Fig. 3. All three plasmids also contained the wild type G at codon 1559 [26] rather than the mutant T which was initially reported for the ER sequence [25]. The T to G transversion shown in Fig. 3 changes codon 351 from GAC to TAC, which changes the predicted amino acid at that position from aspartate to tyrosine.

Confirmation of mutant ER in additional MCF-7/MT2 samples

We wished to determine whether the mutation in the MCF-7/MT2 occurred during the initial development of the tumor as described in the accompanying paper [7], or occurred later and was specific only to the tumor sample shown in Fig. 2. We repeated the SSCP analysis with primer set 4, using the original and two additional MCF-7/MT2 tumor samples (Fig. 4). One of the new samples was from an E_2 treated tumor and the other was from a tumor treated with TAM. All three tumor samples expressed predominantely mutant ER, although faint bands comigrating with the wild type ER (HEGO) were visible in lanes from E_2 and placebo treated samples (Fig. 4, top panel). These wild type bands appeared to be strongest in the sample taken from an E_2 treated tumor.

Discussion

Clinical data accumulated during the past two decades has demonstrated that TAM can be an effective treatment for all stages of breast cancer in both pre- and postmenopausal patients [29–33]. However, since TAM appears to act primarily as a cytostatic and not a cytotoxic agent, residual tumor cells remain in the patient's body held in a growth inhibited state by TAM. Therefore, most patients treated with TAM eventually experience disease recurrence. Some of these recurrences will be insensitive to any form of endocrine intervention, and must be treated with cytotoxic chemotherapy. However, some patients will have second-line responses to other forms of endocrine therapy, indicating that the resistance to TAM is specific, but that the estrogen response machinery is still functional.

There is limited clinical evidence that some patients may develop recurrence because their tumors become TAM stimulated. There are anecdotal reports of patients experiencing objective response after TAM withdrawal [34–36], and one small trial which reported a withdrawal responses in 10% of patients with advanced breast cancer [37]. In addition, we [2, 4] and others [1, 3] have demonstrated the reproducible development of TAM stimulated growth in a laboratory model of human breast cancer, i.e., MCF-7 cells grown as solid tumors in athymic mice.

Several hypotheses have been suggested and tested in an effort to understand the mechanism

leading to TAM stimulated growth in laboratory models. Work from Osborne's laboratory [3, 5, 6] using a similar model system, as well as clinical samples, had suggested that TAM stimulated tumor growth was associated with a decrease in the intracellular levels of TAM and its metabolites [3, 5] and an increase in the conversion of hydroxylated TAM metabolites from the more strongly antiestrogenic (Z) isomers to the weakly antiestrogenic and more strongly estrogenic (E) isomers [3, 5, 6]. We have recently published a report which indicates that neither of these mechanisms can explain TAM stimulated growth in our model system, since neither occurs in our tumor model [4].

Work in this laboratory [14–16] and others [8–13] has shown that alterations in the sequence of a steroid receptor can dramatically alter responses to various ligands, even to the extent of causing the receptor to respond to conventionally antagonistic ligands as agonists. We now report the isolation and sequencing of an estrogen receptor variant expressed by the TAM stimulated tumor variant MCF-7/MT2, which is described in the accompanying report [7]. MCF-7/MT2 tumors implanted in athymic mice are stimulated to grow by either TAM or E_2. We hypothesize that expression of this mutant ER may contribute to this phenotype, especially since the mutation is near a site where another mutation has been shown to confer agonist activity to the antiestrogenic ligands 4-OHT [15] and RU 39,411 [16]. Indeed, recent experiments from our laboratory (Catherino, Wolf, and Jordan, unpublished results) have shown that the ER from the MCF-7/MT2, occupied with either E_2 or 4-OHT, is capable of stimulating luciferase activity from a vitellogenin ERE, whereas wild-type ER mediated expression is not stimulated over control levels by 4-OHT.

Although the mutant ER was the predominant form expressed by MCF-7/MT2 tumor cells, weak bands comigrating with wild type ER bands were also detected in 2 of the 3 samples tested (Fig. 4). It cannot be determined, however, whether the wild type ER was expressed in the same cells as the mutant but at a lower concentration, or if a small number of wild-type ER expressing cells were mixed in with a larger number of tumor cells expressing mutant ER. The latter hypothesis is probably more logical, based on the data in Fig. 4. The highest level of wild-type ER bands was seen in the E_2 treated tumor, with a lesser amount visible in the placebo lane, and little if any detectable in the TAM treated sample. This would be the expected result in a heterogeneous tumor, where cells expressing a wild type receptor would begin to grow again after being switched from TAM to E_2, but would be at a growth disadvantage in a tumor treated continuously with TAM.

Obviously, since only wild type ER was detected in three other TAM stimulated tumors (MCF-7 TAM, MCF-7/MF1 and MCF-7/MT3), other mechanisms not requiring a mutant ER must also be capable of leading to TAM stimulated growth. Alterations in the structure of other transcription factors which interact with the ER in the estrogen response pathway might give rise to proteins capable of interacting equally as well with an ER occupied either by E_2, TAM, or a TAM metabolite. Another possibility does not require alterations in the structure of any proteins, but rather an alteration in the profile of transcription factors expressed by a tumor cell. TAM is known to vary in its agonist versus antagonist activities across different species and indeed even among different tissue types in a single species. TAM acts as an antiestrogen on breast tissue [38] but in the endometrium, liver, and bone (reviewed in [39]) it has much greater agonist activity. If a TAM treated breast tumor cell began expressing a profile of transcription factors more representative of endometrial, hepatic, or bone cells, it would develop the ability to respond more strongly to the partial agonist actions of TAM. Before this hypothesis can be fully investigated, however, the other cellular proteins making up the estrogen responsive transcription complex must be identified and characterized.

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