Published OnlineFirst January 20, 2009; DOI: 10.1158/0008-5472.CAN-08-2806

Research Article

Tamoxifen Induces Expression of Immune Response–Related Genes in Cultured Normal Human Mammary Epithelial Cells

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Abstract

Use of tamoxifen is associated with a 50% reduction in breast cancer incidence and an increase in endometrial cancer incidence. Here, we documented tamoxifen-induced gene expression changes in cultured normal human mammary epithelial cells (strains 5, 16, and 40), established from tissue taken at reduction mammoplasty from three individuals. Cells exposed to 0, 10, or 50 μmol/L of tamoxifen for 48 hours were evaluated for (*E*)-α-(deoxyguanosine-N^2-yl)-tamoxifen (dG-N^2-TAM) adduct formation using TAM-DNA (DNA modified with dG-N^2-TAM) chemiluminescence immunoassay, gene expression changes using National Cancer Institute DNA-oligonucleotide microarray, and real-time PCR. At 48 hours, cells exposed to 10 and 50 μmol/L of tamoxifen were 85.6% and 48.4% viable, respectively, and there were no measurable dG-N^2-TAM adducts. For microarrays, cells were exposed to 10 μmol/L of tamoxifen and genes with expression changes of >3-fold were as follows: 13 genes up-regulated and 1 down-regulated for strain 16; 17 genes up-regulated for strain 5, and 11 genes up-regulated for strain 40. Interferon-inducible genes (*IFITM1, IFIT1, MXI*, and *GIP3*), and a potassium ion channel (*KCNJ1*) were up-regulated in all three strains. No significant expression changes were found for genes related to estrogen or xenobiotic metabolism. Real-time PCR revealed the up-regulation of *IFNA1* and confirmed the tamoxifen-induced up-regulation of the five other genes identified by microarray, with the exception of *GIP3* and *MX1*, which were not up-regulated in strain 40. Induction of IFN-related genes in the three normal human mammary epithelial cell strains suggests that, in addition to hormonal effects, tamoxifen exposure may enhance immune response in normal breast tissue. [Cancer Res 2009;69(3):1150–5]

Introduction

In addition to surgery and radiation therapy, estrogen receptor (ER)–positive breast cancer is frequently treated with adjuvant therapy that may include tamoxifen (Nolvadex), a tamoxifen analogue or an aromatase inhibitor (1–4). Tamoxifen therapy reduces the incidence of contralateral breast cancer in breast cancer survivors by 47% (5), and new breast cancers in women at high-risk (prophylactic use) by 38% (6). However, increases in endometrial (6, 7) and rare uterine cancers (8) in women receiving tamoxifen therapy raises concern for women receiving tamoxifen for long periods of time. This concern is enhanced by reports of a strong hepatocarcinogenic response in tamoxifen-exposed rats, in which both hepatic DNA formation of dG-N^2-TAM (TAM-DNA) adduct (9, 10) and liver tumor incidence (11) correlated with dose, suggesting that classical genotoxicity may be the predominant mechanism for liver tumor formation in this model (10, 12, 13). In women, the mechanism underlying tamoxifen-induced endometrial tumor formation is a topic of some controversy, with some studies indicating a genotoxic mechanism and others implying hormonally controlled events (14–21). An ongoing population-based case-control study (22) may solve this controversy, but the final report has not been published. The investigators compared endometrial cancer incidence in breast cancer survivors receiving tamoxifen and toremifene. Toremifene has been shown to be nongenotoxic in experimental models (23). However, a report documenting similar frequencies of K-*ras* codon 12 mutations in endometrium from women receiving either tamoxifen or toremifene suggests that similar genotoxic events may occur with both treatments (21).

We considered that tamoxifen-induced changes in DNA damage and gene expression may elucidate pathways relevant for molecular mechanisms of drug activity. The current study has focused on normal breast using strains of normal human mammary epithelial cells (NHMEC) derived from human breast tissue taken at reduction mammoplasty from healthy women. In this study, the three different strains, derived from three different individuals, reflect human interindividual variability and similarity with regard to tamoxifen-induced gene expression. Cells were exposed for 48 hours to a tamoxifen concentration similar to that found in human plasma, and there were no measurable TAM-DNA adducts in any NHMEC strain. However, significant changes in gene expression, particularly for immune-response genes, were observed first by microarray and subsequently confirmed by real-time (RT) PCR for the genes of interest. This study provides evidence of a nonhormonal mechanism for tamoxifen activity in human breast.

Materials and Methods

Chemicals. Tamoxifen and calf thymus DNA were obtained from Sigma. Opaque 96-well high binding plates were purchased from Greiner Labortechnik (PGC Scientific). Biotinylated anti-rabbit IgG and streptavidin-alkaline phosphatase were from Applied Biosystems. I-Block (Casein) and CDP-Star with Emerald II were from Applied Biosystems. Reacti-Bind DNA coating solution was obtained from Pierce. Chemiluminescence immunoassay (CIA) wash buffer was obtained from KD Medical. PBS was from Life Technologies. The mammary epithelial cell growth medium (MEGM) bullet kit, serum-free MEGM, and trypsin were purchased from Clonetics. TRIzol was purchased from Invitrogen Life Technologies, cDNA

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doi:10.1158/0008-5472.CAN-08-2806

Published OnlineFirst January 20, 2009; DOI: 10.1158/0008-5472.CAN-08-2806

synthesis was performed using the iScript cDNA Synthesis Kit (Bio-Rad Corp.) and RT-PCR was performed using the SYBR Premix Ex Taq, Perfect Real-time kit (Takara Bio, Inc.).

NHMEC culture, ER status, tamoxifen exposure, and cell survival. Three primary NHMEC strains, M98040 (strain 40), M98016 (strain 16), and M99005 (strain 5) which were described previously (24), were grown in serum-free MEGM (Clonetics). These strains were characterized for ER by immunohistochemical staining. Briefly, cells that were grown in microscope chamber slides were washed in PBS [KH_2PO_4 (1.06 mmol/L), Na_2HPO_4 (5.6 mmol/L), NaCl (154 mmol/L); pH 7.4] and then fixed with ethanol (5 min, −20°C). Fixed cells were thawed at 37°C (0.1% Triton X-100 in PBS) and permeabilized in Triton X-100 (ambient temperature, 30 min). The Triton X-100 was then washed out with PBS and the cells were incubated with a primary anti-ER rabbit IgG (4 μg/mL at 4°C for 16 h; Santa Cruz Biotechnology). The primary antibody was removed by washing thrice with PBS and the cells were incubated in goat serum (37°C for 20 min) before washing again in PBS. The cells were incubated with fluorescein-conjugated goat anti-rabbit (diluted 400-fold; Santa Cruz Biotechnology) at 37°C for 45 min in the dark, then washed thrice with PBS and mounted (M1289, Sigma) for fluorescence microscopy. None of the three strains expressed ERβ, but the strain 16 and strain 40 cells were positive for ERα.

Cells (at passage 7–13) were plated at a density of 1×10^6 cells/15 cm plate or T-175 flask for DNA preparation, and at a density of 1×10^6 cells/six-well plate for RNA preparation and for measuring cell survival. Plated cells were grown for 48 h prior to treatment with either 10 or 50 μmol/L of tamoxifen, or vehicle (DMSO) for an additional 48 h. After 48 h, cells were trypsinized and counted using a Coulter Particle Counter (Model Z1, Coulter Electronics).

For TAM-DNA adduct quantitation, three dishes or flasks of cells were exposed under the same conditions on three separate occasions. For isolation of DNA, the cells were washed twice in PBS and lysed in cell lysis buffer (50 mmol/L Tris-HCl, 0.1 mol/L EDTA, 0.1 mol/L NaCl, 1.0% SDS), and incubated first with RNase A for 1 h at 37°C and then with proteinase K for 1 h at 70°C. The lysate was then extracted once with phenol/chloroform/isoamyl alcohol, and DNA was precipitated with 1.0 mL of ethanol and subsequently resuspended in water. For some studies, DNA was isolated by nonorganic extraction (DNA Extraction Kit, Serologicals Corporation). DNA was quantified by UV spectrophotometry at A_{260}.

For microarray analyses, three replicate exposures were performed for the preliminary study and then confirmed by an independent exposure in duplicate for each cell strain. For isolation of RNA, the cells were lysed with 1.0 mL of TRIzol Reagent (Invitrogen Life Technologies) and RNA was extracted according to the manufacturer's protocol. Residual DNA was removed by digestion with DNase I, and the total RNA quantity and purity were assessed by spectrophotometry and gel electrophoresis, respectively.

For RT-PCR experiments, NHMEC strains were subcultured to passage 6 from frozen stocks and exposed to 10 μmol/L of tamoxifen for 48 h on two separate occasions. cDNA was prepared from RNA, and each cDNA sample was assayed six times by RT-PCR for *IFIT1, IFITM1, MX1, GIP3*, and *KCNJ1*. *IFNA1* was assayed thrice.

TAM-DNA CIA. Rabbit antiserum, elicited against DNA containing 2.4% modification with (E)-α-(deoxyguanosine-N^2-yl)-tamoxifen (dG-N^2-TAM) was used in the TAM-DNA CIA as previously described (25), with additional specific details below. For the TAM-DNA standard curve, we used DNA modified to 4.8 dG-N^2-TAM adducts/10^6 nucleotides, and serial dilutions were carried out to give 6.630 to 0.009 fmol dG-N^2-TAM per well. Competition was achieved by mixing anti–TAM-DNA antiserum with either TAM-DNA standard plus carrier or biological sample DNA in PBS, so that each well contained 5 μg of total DNA. Anti–TAM-DNA was used at a final dilution of 1:1,000,000 in I-Block solution (Applied Biosystems). The final light emission was measured at 542 nm using a TR717 Microplate Luminometer (PE Applied Biosystems). For the TAM-DNA standard curve, 50% inhibition was at 0.89 ± 0.12 fmol dG-N^2-TAM (mean ± SE, n = 5). Because up to 20 μg of DNA could be analyzed, the limit of detection was calculated to be ~0.3 dG-N^2-TAM adducts/10^8 nucleotides.

Microarray analysis of gene expression. cDNA, generated from 20 μg of RNA by Fairplay Kit (Stratagene), was labeled with Cy3 (unexposed control) and Cy5 (tamoxifen-exposed) by indirect coupling, denatured and hybridized to Hs-Operon v2-vB1 oligoarrays containing <20,000 immobilized human gene elements (Microarray Facility, Advanced Technology Center, National Cancer Institute). After overnight hybridization at 42°C, microarrays were scanned on a GenePix4000A scanner and analyzed by the NCI MicroArray Database system. Genes with ≥3-fold color intensity change in >66% of the arrays were considered of interest and subjected to further analysis. For each RNA sample, array data was confirmed once using reciprocal CY3-CY5 labeling. Microarray data has been entered into the GEO system and the MIAME accession number is GSE13476.

RT-PCR. RNA (1.0 μg) was used for cDNA synthesis by the iScript cDNA synthesis kit (Bio-Rad Corp.). All RT-PCR reactions were performed using the MyIQ single color real-time detection system (Bio-Rad Corp.), and RT-PCR was performed using the SYBR Premix Ex Taq, Perfect real-time kit (Takara Inc.) according to the manufacturer's protocols. The primers used for RT-PCR amplification for gene expression quantification are listed in Table 1 and were purchased from Invitrogen Life Technologies.

Statistical analysis. Statistical analysis of the microarray data was performed using the NCI MicroArray Database system, with 66% concordance among assays considered significant. RT-PCR data, for the comparison between untreated and tamoxifen-treated cells, was evaluated using Student's t test.

Results

ER status, cell survival, and TAM-DNA adduct formation. The NHMEC strains used in these studies were designated 40, 16, and 5, and were characterized for ER status. None contained ERβ, but the strain 16 and strain 40 cells were positive for ERα. Unexposed NHMEC strain 40 cells underwent 1.4 population doublings in 48 hours, and by comparison, cells exposed to 10 and 50 μmol/L of tamoxifen had 1.2 and 0.67 population doublings, respectively. These corresponded to 85.6% and 48.4% survival, respectively. Because the toxicity observed with the higher dose was judged unacceptable, the subsequent microarray and RT-PCR studies used 10 μmol/L of tamoxifen.

DNA extracted from the three NHMEC strains, exposed to 0, 10, and 50 μmol/L of tamoxifen for 48 h, was subjected to TAM-DNA

Table 1. Primer sequences for RT-PCR

Gene	Forward primer	Reverse primer
KCNJ1	GTGCCAAGACCATTACGTTC	TAGCCACTCGGATTAGGAGG
IFIT1	TTGCCTGGATGTATTACCAC	GCTTCTTGCAAATGTTCTCC
IFITM1	TCTTCTTGAACTGGTGCTGTC	GTCGCGAACCATCTTCCTGT
MX1	AGGACCATCGGAATCTTGAC	TCAGGTGGAACACGAGGTTC
G1P3	CTGATGAGCTGGTCTGCGAT	TAGCTATGACGACGCTGCTG
IFNA1	TCGCCCTTTGCTTTACTGAT	GGGTCTCAGGGAGATCACAG

Published OnlineFirst January 20, 2009; DOI: 10.1158/0008-5472.CAN-08-2806

CIA and showed no evidence of measurable dG-N^2-TAM adducts. Using up to 20 μg of DNA/well, the limit of detection was 0.3 dG-N^2-TAM adducts/10^8 nucleotides.

Microarray studies in NHMEC strains exposed to 10 μmol/L of tamoxifen for 48 hours. Microarray analyses, performed using the NCI microarray system, used RNA/cDNA samples obtained from three independent exposures for each cell strain. Each RNA/cDNA sample was assayed on 7 to 12 microarrays, at least one of which involved reciprocal labeling for microarray confirmation. The data primarily showed up-regulation of genes in tamoxifen-exposed cells compared with unexposed cells. We chose to evaluate only genes that were up-regulated or down-regulated by ≥3-fold, and a list of those genes is shown in Table 2. One notable conclusion that can be drawn from Table 2 is that many of the genes that are the most highly up-regulated by tamoxifen seem to be immune response–related genes, associated either with IFN regulation, inflammation, histocompatibility or additional responses to external insult and stress. The specific microarray data for cells altered by ≥3-fold are shown in Tables 3, 4, and 5, for strains 16, 5, and 40, respectively. All of the genes altered significantly were up-regulated, with the exception of *SLC7A5*, which was down-regulated. The cell strains can be ranked for magnitude of up-regulation in the following order: strain 16 > strain 5 > strain 40. Genes which were up-regulated by microarray in all three cell strains included *IFIT1, IFITM1, MX1, GIP3*, and *KCNJ1*.

RT-PCR of genes up-regulated in all three NHMEC strains by exposure to 10 μmol/L of tamoxifen for 48 hours. Because the microarray is essentially a screening procedure, it was important to confirm the microarray results with RT-PCR. Primers were designed and RT-PCR was performed for the five genes up-regulated in all three NHMEC strains: *IFIT1, IFITM1, MX1, GIP3* and *KCNJ1*. IFNα (*IFNA1*) was not present on the NCI microarrays used here, but the primers were developed and the expression of this gene was also assayed by RT-PCR. The results are presented in Tables 3, 4, and 5 (*last column*).

For the six genes examined by RT-PCR, there was up-regulation that generally compared well with the microarray data. Similar to the results of the microarray analysis, strain 16 had the greatest increase in gene expression, followed by strain 5, and strain 40. In addition, in all three NHMEC strains, the levels of up-regulation observed with *IFITM1, IFIT1*, and *KCNJ1* were greater than those observed with *MX1* and *GIP3* (Tables 3, 4, and 5). When examined by RT-PCR, NHMEC strain 40 showed no up-regulation for *MX1* and *GIP3* (Table 5). In NHMEC strains 16, 40, and 5, the up-regulation observed for *IFNA1* was 7-fold, 5-fold, and 13-fold, respectively, very much in the same range as the up-regulation of the IFN-inducible genes *IFIT1* and *IFITM1*.

Discussion

In this study, we exposed NHMEC strains to 10 and 50 μmol/L of tamoxifen for 48 hours to investigate TAM-DNA adduct formation and tamoxifen-induced alterations in gene expression. TAM-DNA adduct formation was not measurable by TAM-DNA immunoassay, but changes in gene expression determined by microarray and

Table 2. Genes with expression changes of ≥3-fold found by NCI microarray in one or more of the NHMEC strains (5, 16, and 40) evaluated after exposure to 10 μmol/L of tamoxifen for 48 h

Gene	Name	Function
cig5	Viperin	Unknown; similar to inflammatory response protein 6
BST2	Bone marrow stromal cell antigen 2	Unknown; pre–B-cell growth
TRIM22	Tripartite motif containing 22	Down-regulates transcription from HIV-1 LTR promoter
SPP1	Secreted phosphoprotein 1 (osteopontin)	Target of p53 with role in osteoclast adhesion
OAS3	2′,5′ oligoadenylate synthase 3	Catalyzes 2′-5′ oligomers of dA to bind/activate RNase L; inhibits cell protein synthesis and viral infection resistance
OAS1	2′,5′ oligoadenylate synthase 1	Catalyzes 2′-5′ oligomers of dA to bind/activate RNase L
KCNJ1	Potassium inwardly rectifying channel	K2+ homeostasis, Bartter syndrome (salt wasting, low blood pressure)
C1orf29	Chr 1 ORF 29	Histocompatibility 28
B2M	β-2-microglobulin	β-chain of MHC
IFITM1	IFN-induced transmembrane protein 1	Cell growth control; involved in transduction signaling for antiproliferation and homotypic adhesion
G1P3	IFN-α–inducible protein	Unknown; membrane protein?
WARS	Tryptophanyl-tRNA synthetase	Aminoacyl tRNA catalyze amino-acylation of tRNA with tryptophan.
STAT1	Signal transducer and activator of txc	Txc activation; important for cell viability in response to different cell stimuli and pathogens
IFIT1	IFN-induced protein with tetra-tricopeptide repeats 1	Unknown
MX1	Myxovirus resistance 1	Similar to mouse protein that protects against flu infection
IFIT4	IFN-induced protein	Unknown
IFI27	IFN-α–inducible protein 27	Unknown
THBS1	Thrombospondin 1	Adhesive glycoprotein; cell/cell or cell/matrix interactions
LGalS3BP	Lectin, galactoside-binding, soluble	Modulates cell/cell or cell/matrix interactions
SLC7A5	Solute carrier family 7	Unknown

NOTE: All genes were up-regulated, with the exception of *SLC7A5*, which was down-regulated.

Table 3. Gene expression changes (≥3-fold) examined by NCI microarray and RT-PCR in NHMEC 16 cells exposed for 48 h to 10 μmol/L of tamoxifen, compared with unexposed NHMEC strain 16 cells

Gene	Microarray mean $\log_2 \pm$ SD	Microarray mean fold change	Number of microarrays	RT-PCR mean fold change*, †
IFITM1	4.68 ± 1.30	25.8	9	20.8 ± 3.7 †
KCNJ1	4.04 ± 2.22	16.4	9	4.4 ± 1.8 †
IFIT1	4.01 ± 1.71	16.2	9	15.6 ± 5.0 †
IFIT4	3.30 ± 1.80	9.9	7	NA
GIP3	3.03 ± 0.99	8.2	8	3.3 ± 1.1
MX1	2.82 ± 1.18	7.0	9	9.5 ± 2.0 †
IFI27	2.57 ± 1.11	5.9	9	NA
STAT1	2.55 ± 0.87	5.7	8	NA
BST2	2.50 ± 0.55	5.7	6	NA
OAS3	2.47 ± 0.90	5.6	6	NA
HLA-C	2.23 ± 0.83	4.7	9	NA
B2M	2.05 ± 0.52	4.1	9	NA
LGALS3BP	1.18 ± 0.72	2.3	9	NA
IFNA1 ‡	NA			7.1 ± 2.3 †
SLC7A5	−1.75 ± 0.54	0.3	9	NA

Abbreviation: NA, no assay.
*Each cDNA was assayed six times by RT-PCR, except for *IFNA1*, which was assayed thrice.
† $P < 0.05$.
‡ *IFNA1* was not printed on the original microarray.

confirmed by RT-PCR showed the up-regulation of a series of immune response/IFN pathway genes in each of the three normal mammary epithelial cell strains.

We used NHMEC strains designated 5, 16, and 40 that were derived from tissue taken at reduction mammoplasty from three different individuals. Strains 16 and 40 were positive for ERα, and all three strains were negative for ERβ. By microarray, we found that, after 48 hours of exposure to a plasma-equivalent tamoxifen dose, 1 gene was down-regulated ≥3-fold, and 19 genes were up-regulated ≥3-fold. Most of the up-regulated genes were immune-response–related genes, and there were no alterations in xenobiotic metabolism or hormone-responsive genes. The most common changes were found in histocompatibility genes and intermediates in the JAK/STAT-IFN signal transduction pathway (26, 27), and because all three cell strains showed remarkably similar patterns of gene up-regulation, it seems that these gene expression changes may constitute a relatively common early stress response to tamoxifen exposure in NHMECs.

The importance of these immune-related pathways was also shown in a murine, human mammary carcinoma xenograft model by Becker and colleagues (28). The authors cultured human tamoxifen-sensitive MaCa 3366 breast ductal carcinoma cells for

Table 4. Gene expression changes (≥3-fold) examined by NCI microarray and RT-PCR in NHMEC 5 cells exposed for 48 h to 10 μmol/L of tamoxifen, compared with unexposed NHMEC strain 5 cells

	Microarray mean $\log_2 \pm$ SD	Microarray mean fold change	Number of microarrays	RT-PCR mean fold change*, †
cig5	3.72 ± 1.33	13.2	13	NA
IFIT1	3.33 ± 1.17	10.1	14	6.8 ± 2.0 †
IFITM1	3.19 ± 0.92	9.1	14	7.6 ± 1.1 †
KCNJ1	3.04 ± 1.48	8.2	14	16.3 ± 12.4 †
C1orf29	2.41 ± 0.73	5.3	14	NA
GIP3	2.16 ± 0.51	4.5	14	3.0 ± 1.5
MX1	2.12 ± 0.89	4.4	14	6.0 ± 2.4 †
BST2	2.50 ± 0.55	3.6	14	NA
IFNA1 ‡	NA			13.1 ± 6.1 †

Abbreviation: NA, no assay.
*Each cDNA was assayed six times by RT-PCR, except for *IFNA1*, which was assayed thrice.
† $P < 0.05$.
‡ *IFNA1* was not printed on the original microarray.

Published OnlineFirst January 20, 2009; DOI: 10.1158/0008-5472.CAN-08-2806

Table 5. Gene expression changes (≥3-fold) examined by NCI microarray and RT-PCR in NHMEC strain 40 cells exposed for 48 h to 10 μmol/L of tamoxifen, compared with unexposed NHMEC strain 40 cells

Gene	Microarray mean log_2 ± SD	Microarray mean fold change	Number of microarrays	RT-PCR mean fold change*, †
cig5	3.11 ± 0.82	8.7	10	NA
IFITM1	3.09 ± 0.49	8.5	10	5.6 ± 2.9 †
SPP1	2.90 ± 0.41	7.5	10	NA
KCNJ1	2.49 ± 0.87	5.6	12	3.5 ± 1.5 †
IFIT1	2.42 ± 0.65	5.3	10	4.4 ± 1.3 †
MX1	2.07 ± 0.65	4.2	12	0.5 ± 0.1
GIP3	2.03 ± 0.73	4.1	12	0.6 ± 0.1
IFNA1 ‡	NA			5.2 ± 1.2 †

Abbreviation: NA, no assay.
*Each cDNA was assayed six times by RT-PCR, except for *IFNA1*, which was assayed thrice.
† $P < 0.05$.
‡ *IFNA1* was not printed on the original microarray.

2 years in the presence of tamoxifen, to develop a tamoxifen-resistant version (MaCa 3366/TAM) of this tumor. Both tumor lines were transplanted into nude mice and gene expression was compared in the presence and absence of additional tamoxifen exposure using the Affymetrix microarray. These authors showed up-regulation of 9 IFN-related genes in tamoxifen-resistant human MaCa 3366 cells exposed to tamoxifen; these included, *BST2, IFITM2, GIP2, GIP3, IFITM1, LGALS3BP, IFIT1, MX1,* and *IFI27*. Becker and colleagues (28) also reported differential expression of some estrogen-responsive genes, which was not reproduced in this study.

Several studies using cultured cells have reported tamoxifen-induced alterations in gene expression for the IFN-regulated JAK/STAT pathway. Itoh and colleagues (29) used ER-positive MCF-7 cells that were transfected with the aromatase gene and exposed for 7 days to tamoxifen in the presence of androgen. They reported modest increases in expression for some of the same STAT1 pathway genes seen in our study, including *GIP2, IFI27*, and *IFIT1*. Perou and colleagues (30) found up-regulation of genes in this pathway, including *STAT1, OAS1*, and *IFI17*, and postulated that *STAT1* up-regulation was present at all stages of cell growth. Similarly, we found up-regulation of *STAT1, OAS1, OAS3*, and *IFI27*. In a subsequent study, Perou and colleagues (31) reported substantial variation among primary human breast tumors for genes related to the *STAT1* signal transduction pathway, suggesting that expression of IFN and related events may comprise an important pathway in normal breast tissue with and without tamoxifen, as well as in breast tumors.

Several studies investigating gene expression patterns in MCF-7 breast cancer cells, with or without tamoxifen exposure, did not report alterations in genes related to the JAK/STAT signal transduction pathway. Using MCF-7 cells exposed to tamoxifen, Gadal and colleagues (32) showed altered expression of genes associated with cytoskeletal modeling, DNA repair, active ER formation, growth factor synthesis and mitogenic pathways. Frasor and colleagues (33) found up-regulation of 50 genes in tamoxifen-exposed ER-positive MCF-7 cells, and Hodges and colleagues (34) found expression changes in cell cycle–related genes in ER-positive MCF-7 cells exposed to 4-hydroxy-tamoxifen. It is apparent that different studies queried different numbers of genetic elements, and it is not clear if our genes of interest were always examined.

It is likely that gene expression data obtained from a cancer cell line, such as MCF-7 cells, or from human breast cancer tissue (35, 36), will have different expression patterns than normal human breast tissue or cultured breast epithelial cells. In performing these experiments with NHMECs, we attempted to model events occurring in normal breast tissue in order to focus on tamoxifen-induced alterations in gene expression. One conclusion that can be drawn from these studies is that, whereas ER status is not the same in all three of these NHMEC strains, the tamoxifen-induced gene expression patterns were so similar, in cells that were ERα-positive and ERα-negative, that these particular changes seemed to be independent of ER status.

In addition to the classic immune response mediation, IFNs have long been known to have static and chemotherapeutic effects on tumor cells (26, 37). IFNs, given in conjunction with tamoxifen, enhanced growth inhibition in various human tumor cell lines, including estrogen-dependent and estrogen-independent MCF-7 breast cancer xenografts (38, 39), and six additional hormone-dependent and hormone-resistant breast cancer cell lines (40). Underlying mechanisms seem to include the induction of apoptosis (41, 42), with participation of the IFN regulatory factor-1 and/or thioredoxin reductase. The induction of IFN-associated genes in NHMECs exposed to tamoxifen in this study suggests that, in addition to the known hormonal mechanisms, tamoxifen may act to inhibit the appearance of nascent breast tumors by inducing some IFN-related genes and possibly enhancing apoptosis. Alternatively, the products of IFN-related gene expression in normal tissue may have an inhibitory effect on the growth of neighboring nascent tumor cells. Current literature supports the contention that tamoxifen induces the expression of JAK/STAT pathway intermediates, and available studies suggest that there may be important nonhormonal mechanisms of tamoxifen activity in normal breast tissue.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 7/22/2008; revised 10/10/2008; accepted 10/11/2008.

Grant support: Intramural program of the NIH, NCI, Center for Cancer Research and used cell strains developed at the National Institute for Occupational Safety and Health.

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We wish to thank Ms. K. Meyer for microarray GEO submissions.

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Cancer Res 2009;69:1150-1155. Published OnlineFirst January 20, 2009.