Abstract

Context: The estrogen receptor (ER) status in breast cancer plays a major role in the progression and metastatic potential of breast cancer in women. Breast cancer cells lacking the ER are usually more advanced and more difficult to treat than ER+ breast cancer cells. ER- women have more advanced breast cancer at the time of diagnosis than ER+ women. ER- breast cancer cells in women, regardless of age, are more likely to have tumor Grade III or IV with fewer Grade I and II tumor stages combined for each individual stage group. Studies have suggested a strong correlation between fat intake and the elevated risk of ER+ breast cancer cells. Materials and Methods: We studied the role of ER status on the gene expression in breast cancer cells in response to omega-3 and omega-6 fatty acids using microarrays. We have studied gene expression patterns in 8 breast cancer cell lines (4 ER- and 4 ER+) in response to Eicosapentanoic (EPA) and Arachidonic (AA) acids. Statistical Analysis: Analysis of Variance (ANOVA) t-test analysis was carried out to identify genes differentially expressed between the two groups. Results: We identified genes which were significantly correlated with the ER status when breast cancer cells were treated with these fatty acids. Conclusion: We have determined ER-related gene expression patterns in breast cancer cells in response to fatty acids. Additional studies of these biomarkers may enlighten the importance of the ER status on the mechanistic and therapeutic roles of fatty acids in breast cancer.

Keywords: Estrogen receptor, breast cancer, microarray, fatty acids, omega-3, omega-6

INTRODUCTION

Estrogens control the growth and differentiation of mammary glands and regulate gene expression in breast cells through the estrogen receptor (ER). ERs are expressed in 70% of breast cancer cases where cancer cell growth is controlled by estrogen and is often susceptible to treatment with inhibitors that block the interaction between estrogen and the estrogen receptor.

The estrogen receptor status in breast cancer plays a major role in the progression and metastatic potential of breast cancer in women. Breast cancer cells lacking the (ER-) are usually more advanced and more difficult to treat than ER+ breast cancer cells. A disparity in breast carcinoma survival between ER- and ER+ cases has been noted over the past several decades. ER- women have more advanced breast cancer at the time of diagnosis than ER+ women. In addition, ER- women tend to have breast cancer tumor types that are more aggressive and have poorer prognosis. ER- breast cancer cells in women, regardless of age, are more likely to have tumor grade III or IV with fewer grade I and II tumor stages.
combined and for each individual stage group.

Epidemiologic studies have found a significant correlation between ER+ breast cancer cells and several lifestyle risk factors, such as higher body mass index, earlier age at menarche, nulliparity, and diet.\(^1\) Cho et al. studied the association between dietary fat intake and breast cancer in premenopausal women and found a strong correlation between fat intake and the elevated risk of ER+ breast cancers.\(^3\)

A case-case study that evaluated the association of dietary fat intake of selected fatty acids found that high intakes of linoleic acid in premenopausal breast cancer patients were associated with a threefold higher risk of ER- than ER+ tumors.\(^5\)

The disparities observed in incidence trends and age at diagnosis highlight the need for further investigation of the differences between ER- and ER+ breast cancer cells. Gruvberger et al. studied gene expression profiles in ER- and ER+ breast tumors using microarrays and showed that they had very distinct gene expression patterns.\(^6\) The study found a significant increase in the expression levels of P-cadherin, C/EBP \(\beta\) transcription factor, and ladinin in ER+ breast cancer cells. It also identified GATA3, Cyclin D1 and carbonic anhydrase XII expression to be associated with ER+ breast cancer samples.

In a previous study, we characterized the transcriptional profiles in breast cancer cells treated with omega-3 and omega-6 fatty acids.\(^7\) In that study, we observed differences in gene expression between ER+ and ER- cells in response to the fatty acids, but this was a preliminary finding since only 2 cell lines of each ER status were used; therefore we doubled the number of each group in order to identify gene expression profiles directly associated with ER status. We are now able to describe in more detail the role of ER status on the gene expression in breast cancer cells in response to omega-3 and omega-6 fatty acids using the 4 well-characterized ER- and 4 ER+ breast cancer cells. We identified the genes that were significantly correlated with the ER status when breast cancer cells were treated with these fatty acids.

Note: microarray data have been submitted to the Gene Expression Omnibus (GEO) and can be searched using the Platform ID: GPL8144, Series: GSE14679.

**MATERIALS AND METHODS**

ER- (HCC-1806, MDA-MB-468, Hs578T and SK-BR-3) and ER+ (HCC-70, MCF-7, HCC-1500 and CAMA-1) breast cancer cell lines as well as culture media were obtained from ATCC (Manassass, VA). Fatty acids were obtained from BioMol (Plymouth Meeting, PA). Each fatty acid was aliquoted and aliquots were stored at -70°C until used. The TRIzol™ reagent was obtained from Invitrogen (Carlsbad, CA), iScript cDNA synthesis kit from Bio-Rad (Hercules, CA) and the Micromax Tyramide Signal Amplification (TSA) and Labeling Kit from Perkin Elmer, Inc. (Wellesley, MA).

Cell lines were cultured in the recommended media. Twenty four hours prior to treatment with fatty acids, culture media were removed and cells were washed with PBS and incubated in the same media supplemented with 1% (v/v) insulin/sodium selenite and 1% (v/v) non-essential amino acids in the absence of FBS. At the scheduled times, selected flasks were treated with 10 μM fatty acids added to fresh media and incubated for six and 24 hours respectively. Control cells were incubated in fresh media in the absence of fatty acids.

Total RNA was isolated using TRIzol reagent (Invitrogen, CA) following the manufacturer’s protocol. RNA quality and quantity were determined on an Agilent 2100 Bioanalyzer (Agilent Technologies, CA).

Human cDNA microarrays were prepared as described in Hammamieh et al.\(^8\) Briefly, we used sequence verified oligos (~36,000 oligos) representing the whole genome (Operon, Inc, Huntsville, AL). The oligos were deposited in 3X saline sodium citrate (SSC) at an average concentration of 165 μg/ml on CMT-GAPS II aminopropyl silane-coated slides (Corning, NY), using a VersArray microrarrayer (Bio-Rad, Inc). Arrays were post processed using UV-cross linking at 1200 mJ/cm² and by baking for four hours at 80 °C. Positively charged amine groups (on the slide surface) were then treated with succinic anhydride and N-methyl-2-pyrrolidinone.

Microarray slides were labeled using Micromax Tyramide Signal Amplification (TSA) Labeling and Detection Kit (Perkin Elmer, Inc., MA) as described in Hammamieh et al. Slides were hybridized for 16 hours at 60 °C. Hybridized slides were scanned using GenePix Pro 4000B optical scanner (Axon Instruments, Inc., CA). Intensities of the scanned images were digitalized through Genepix 6.0 software.

Assessment of the overall integrity of the microarray experiment were carried out as described in Hammamieh et al.\(^9\)

Microarray images were visualized and normalized using ImaGene 6.0 (BioDiscovery, Inc., CA); and data was analyzed using GeneSpring 10.1 (Agilent Technologies, CA).

Background and foreground pixels of each spot were segmented using ImaGene (BioDiscovery Inc., CA), and the
highest and lowest 20% groups of the probe intensity were discarded. Local background correction was applied to each individual spot. The genes that passed this filter in all the experiments were further analyzed.

Data filter and statistical analysis were carried out using GeneSpring 10.1. Local background was subtracted from individual spot intensity and genes that failed ‘background check’ in any of the experiments were eliminated from further analysis. Each chip was next subjected to intra-chip normalization (LOWESS). Differentially regulated genes (between control and treated sample sets) were selected using t-test analysis (P < 0.05).

Principal component analysis (PCA) was performed over the given dataset, classifying each sample as a statistical variable in order to confirm the extent of variability within the sample classes and among the pre-designed groups. A two-dimensional hierarchal clustering calculation using Pearson correlation around zero was also performed.

We randomly selected genes to confirm their expression profiles using real time PCR. These genes are Protocadherin, thyroid hormone receptor-associated protein complex component (TRAP150), Mitochondrial ribosomal protein L43, transducer of ERBB2, WNT-2B Isoform 1 oncogene and coiled-coil domain containing 61 (CCDC61). Primer3, A web-based primer designing tool, was used to design primers for selected genes (http://www.frodo.wi.mit.edu/). The specificity of each primer sequence was confirmed by running a blast search. Reverse transcription and Real-time PCR reactions were carried out using iScript cDNA synthesis kit from Bio-Rad (Hercules, CA) and a Real-time PCR kit (Roche, IN), respectively. Each reaction was run in I-Cycler (Bio-Rad, CA) using five technical duplicates. Each sample was also amplified using primer sets for the 18S house-keeping probe of the experiment. The resultant cycle threshold data from each real-time-PCR ‘run’ was converted to fold-change.

**RESULTS**

We have studied gene expression profiles and identified genes differentially expressed between ER- and ER+ breast cancer cells treated with EPA.

Data were normalized by applying inter-chip and intra-chip normalizations using GeneSpring 10.1, as described in the methods section. When we used One-way ANOVA with a P-value < 0.05 we identified 819 genes, out of the 36000 genes, to be differentially expressed between ER- and ER+ breast cancer cells in response to treatment with EPA [Figure 1].

To functionally classify the genes associated with the ER status in breast cancer cells treated with EPA, we used GeneSpring 10.1 and FATIGO+.[9, 10] We have also used Ingenuity Pathway Analysis and GeneCite to carry out...
A detailed pathway analysis using the Biocarta pathways,[11] functional classification of upregulated genes revealed that genes involved in the G2/M DNA damage checkpoint regulation, protein ubiquitination and apoptosis signaling were upregulated in ER+ cells in response to EPA while the cyclin dependent kinase signaling cascade was associated with ER- cells.

Ingenuity analysis of the genes upregulated in ER+ cells identified an apoptosis-related network as being significantly enriched and among the top ranked networks. Some of these genes included caspases and STAT1. They are listed in Table 1 and a simplified network is depicted in Figure 2. 

Some of the genes that were downregulated in ER+ cells in response to EPA were genes involved in β-catenin signaling and the BCL-2 anti-apoptosis pathway [Figure 3]. Table 3 lists the name and the regulation patterns of genes involved in the β-catenin pathway.

The genes of amino acid synthesis pathway were highly enriched in the list of genes downregulated by EPA in ER- cells. These genes are listed in Table 4. We have studied gene expression profiles and identified genes differentially expressed between ER- and ER+ breast cancer cells treated with AA.

When we used One-way ANOVA with a P-value < 0.05 we identified 437 genes to be differentially expressed between ER- and ER+ breast cancer cells in response to treatment with AA [Figure 4].

Functional annotation of the genes differentially upregulated in ER- and ER+ cells showed that ERK/MAPK, NF-κB, EGF

| Table 2: HIF Pathway related genes up regulated in ER+ breast cancer cells in response to EPA |
|-----------------------------------------------|--------------------------|------------------|
| Symbol       | Entrez gene name            | Fold change |
|---------------|--------------------------|------------------|
| ARNT          | aryl hydrocarbon receptor nuclear translocator | 2.0 |
| SFRS1         | splicing factor, arginine/serine-rich 1 | 1.2 |
| DDX5X         | DEAD (Asp-Glu-Ala-Asp) box polypeptide 58 | 5.0 |
| DSG1          | desmoglein 1 | 2.7 |
| PABPC1        | poly(A) binding protein, cytoplasmic 1 | 1.5 |
| PAWR          | PRKC, apoptosis,WT1, regulator | 4.0 |
| PHIP          | pleckstrin homology domain interacting protein | 2.1 |
| RAD21         | RAD21 homolog (S. pombe) | 2.0 |
| RNF7          | ring finger protein 7 | 1.3 |
| RPL36         | ribosomal protein L36 | 1.2 |
| STAT1         | signal transducer and activator of transcription 1, 91kDa | 1.5 |
| TAC1          | tachykinin, precursor 1 | 1.6 |
| WAPAL         | wings apart-like homolog (Drosophila) | 1.9 |
|               |             |                 |
|               |             |                 |

| Table 1: Apoptosis related genes up regulated in ER+ breast cancer cells in response to EPA |
|-----------------------------------------------|--------------------------|------------------|
| Symbol       | Entrez gene name            | Fold change |
|---------------|--------------------------|------------------|
| CASP4         | caspase 4, apoptosis-related cysteine peptidase | 3.5 |
| CLSPN         | clasin homolog (Xenopus laevis) | 1.8 |
| DDX5X         | DEAD (Asp-Glu-Ala-Asp) box polypeptide 58 | 5.0 |
| DSG1          | desmoglein 1 | 2.7 |
| PABPC1        | poly(A) binding protein, cytoplasmic 1 | 1.5 |
| PAWR          | PRKC, apoptosis,WT1, regulator | 4.0 |
| PHIP          | pleckstrin homology domain interacting protein | 2.1 |
| RAD21         | RAD21 homolog (S. pombe) | 2.0 |
| RNF7          | ring finger protein 7 | 1.3 |
| RPL36         | ribosomal protein L36 | 1.2 |
| STAT1         | signal transducer and activator of transcription 1, 91kDa | 1.5 |
| TAC1          | tachykinin, precursor 1 | 1.6 |
| WAPAL         | wings apart-like homolog (Drosophila) | 1.9 |
signaling and VEGF signaling cascades were highly enriched in ER+ cells treated with Arachidonic acids while in ER- cells RAR Activation cascade, IL-4 signaling, insulin receptor signaling, and p53 signaling were significantly expressed.

Functional annotation and pathway analysis of genes up regulated mainly in ER+ breast cancer cells in response to Arachidonic acid show that the top ranked pathway was the ERK/MEK signaling pathway [Figure 5]. Table 5 lists the genes from the ERK- pathway that were up regulated by Arachidonic acid in ER+ breast cancer cells.

We carried out functional annotation for the genes that were up regulated in ER- cells when treated by Arachidonic acid
Figure 2: Ingenuity pathway analysis and expression profiles of genes involved in apoptosis that were uniquely up regulated in ER+ cells in response to EPA. Cells were incubated with EPA for 6 and 24 hrs. RNA samples were isolated and hybridized on the cDNA microarray slides as detailed in materials and methods. Images were analyzed using GenePix 6.0 and data were analyzed using GeneSpring 10.1.

Figure 3: β-catenin cascade that was uniquely down regulated in ER+ cells in response to EPA. Cells were incubated with EPA for 6 and 24 hrs. RNA samples were isolated and hybridized on the cDNA microarray slides as detailed in materials and methods. Images were analyzed using GenePix 6.0 and data were analyzed using GeneSpring 10.1.
and found that the Insulin Receptor signaling pathway was highly enriched. Vascular endothelial growth factor and superoxide dismutase were also up regulated in ER- cells [Figure 6]. Table 6 lists the insulin receptor pathway genes that were uniquely up regulated in ER- cells.

Of the genes that were down regulated by AA uniquely in ER+ cells are genes involved in apoptosis such as Caspase 7, Caspase 9, Caspase 10, TNF, and BCL-2 associated agonist of cell death [Figure 7].

Table 5: ERK pathway related genes up regulated in ER+ breast cancer cells in response to AA

| Symbol | Entrez Gene Name | Fold Change |
|--------|-----------------|-------------|
| ABI1   | abl-interactor 1 | 2.9         |
| AKAP1  | A kinase (PRKA) anchor protein 1 | 2.6 |
| ARHGEF7| Rho guanine nucleotide exchange factor (GEF) 7 | 3.0 |
| BAD    | BCL2-associated agonist of cell death | 2.1 |
| CCDC6  | coiled-coil domain containing 6 | 2.7 |
| CRP    | C-reactive protein, pentraxin-related | 2.8 |
| CX3CL1 | chemokine (C-X3-C motif) ligand 1 | 2.3 |
| CXCR4  | chemokine (C-X-C motif) receptor 4 | 2.4 |
| EGF    | epidermal growth factor (beta-urogastrone) | 2.0 |
| EGFR   | epidermal growth factor receptor | 3.4 |
| ELF3   | E74-like factor 3 (ets domain transcription factor, epithelial-specific) | 2.4 |
| EPHB4  | EPH receptor B4 | 2.1 |
| FGF    | c-fos induced growth factor (vascular endothelial growth factor D) | 2.4 |
| GAB1   | GRB2-associated binding protein 1 | 2.5 |
| GIT2   | G protein-coupled receptor kinase interacting with ArfGAP 2 | 2.9 |
| HSD1   | heat shock 60kDa protein 1 (chaperonin) | 2.4 |
| KLF5   | Kruppel-like factor 5 (intestinal) | 3.9 |
| MATK   | megakaryocyte-associated tyrosine kinase | 2.9 |
| MAM    | melanoma cell adhesion molecule | 2.8 |
| MUC1   | mucin 1, cell surface associated | 3.9 |
| NCK1   | NCK adaptor protein 1 | 3.1 |
| P2RY6  | pyrimidinergic receptor P2Y, G-protein coupled, 6 | 2.7 |
| PIK3CB | phosphoinositide-3-kinase, catalytic, beta polypeptide | 2.6 |
| PIK3R3 | phosphoinositide-3-kinase, regulatory subunit 3 (gamma) | 3.9 |
| PTPN4 | protein tyrosine phosphatase 1, catalytic subunit, alpha isoform | 2.5 |
| PTEN   | protein tyrosine phosphatase 1B | 3.0 |
| PTPN12 | protein tyrosine phosphatase, non-receptor type 12 | 4.3 |
| PXN    | paxillin | 10.8 |
| RHO    | ras homolog gene family, member U | 2.3 |
| RPS27a | ribosomal protein S27a | 2.7 |
| RPS6KA1| ribosomal protein S6 kinase, 90kDa, polypeptide 1 | 2.4 |
| SH3KBP1| SH3-domain kinase binding protein 1 | 2.9 |
| SH2B1  | SH2B1 binding protein 1 | 2.3 |
| SORBS2 | sorbin and SH3 domain containing | 2.0 |
| SOS1   | son of sevenless homolog 1 (Drosophila) | 3.6 |
| STAT3  | signal transducer and activator of transcription 3 (acute-phase response factor) | 2.1 |
| TMOD1  | tropomodulin 1 | 5.0 |
| TRIM29 | tripartite motif-containing 29 | 11.9 |

Table 6: Insulin receptor pathway related genes up regulated in ER- breast cancer cells in response to AA

| Symbol | Entrez gene name | Fold change |
|--------|-----------------|-------------|
| ADIPOQ | adiponectin, C1Q and collagen domain containing | 3.4 |
| BTK    | Bruton agammaglobulinemia tyrosine kinase | 54.9 |
| CDK2   | cyclin-dependent kinase 2 | 2.4 |
| CSF3R  | colony stimulating factor 3 receptor (granulocyte) | 2.1 |
| CTGF   | connective tissue growth factor | 2.1 |
| CTSD   | cathepsin D | 2.2 |
| ESR2   | estrogen receptor 2 (ER beta) | 2.5 |
| GRB2   | growth factor receptor-bound protein 2 | 2.3 |
| HAS2   | hyaluronan synthase 2 | 2.0 |
| HBEFG  | heparin-binding EGF-like growth factor | 2.0 |
| IGHEM  | immunoglobulin heavy constant mu | 2.4 |
| IL4    | interleukin 4 | 3.1 |
| IL6ST  | interleukin 6 signal transducer (gp130, oncostatin M receptor) | 2.5 |
| INSR   | insulin receptor | 43.5 |
| IRF4   | interferon regulatory factor 4 | 2.1 |
| KHDRBS1| KH domain containing, RNA binding, signal transduction associated 1 | 4.6 |
| LIFR   | leukemia inhibitory factor receptor alpha | 2.5 |
| LOX    | lystate oxidase | 2.3 |
| LTA    | lymphotixin alpha (TNF superfamily, member 1) | 2.0 |
| LTB    | lymphotixin beta (TNF superfamily, member 3) | 2.0 |
| NR3C1  | nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) | 2.1 |
| NRG1   | neuregulin 1 | 4.5 |
| NTF3   | neurotrophin 3 | 2.5 |
| PIK3R1 | phosphoinositide-3-kinase, regulatory subunit 1 (alpha) | 3.5 |
| PTEN   | phosphatase and tensin homolog | 2.3 |
| RAC1   | regulator of calcineurin 1 | 2.9 |
| SMAD4  | SMAD family member 4 | 3.7 |
| SOD2   | superoxide dismutase 2, mitochondrial | 3.8 |
| SPIB   | Spi-B transcription factor (Spi-1/PU.1 related) | 2.4 |
| TRAF4  | TNF receptor-associated factor 4 | 2.2 |
| VEGFA  | vascular endothelial growth factor A | 2.5 |
| VPREBI | pre-B lymphocyte 1 | 4.5 |
Figure 4: A pseudo color cluster view of genes differentially expressed between ER- and ER+ breast cancer cells in response to AA. Cells were treated with AA at 6 and 24 hrs. RNA was isolated and hybridized on the cDNA microarray slides as detailed in materials and methods. Images were analyzed using GenePix 6.0 and data were analyzed using GeneSpring 10.1.

Figure 5: Ingenuity pathway analysis of genes up regulated in ER+ cell in response to AA shows that the ERK/MEK pathway was significantly associated with ER+. Cells were treated with AA at 6 and 24 hrs. RNA was isolated and hybridized on the cDNA microarray slides as detailed in materials and methods. Images were analyzed using GenePix 6.0 and data were analyzed using GeneSpring 10.1.
Figure 6: The insulin receptor cascade was uniquely up regulated in ER- cells when treated with AA. Ingenuity pathway analysis of the insulin receptor cascade showing the expression patterns of the pathway component in ER- cells. Cells were treated with AA at 6 and 24 hrs. RNA was isolated and hybridized on the cDNA microarray slides as detailed in materials and methods. Images were analyzed using GenePix 6.0 and data were analyzed using GeneSpring 10.1.

Figure 7: Pathway analysis of genes involved in cell-cell signaling significantly associated with ER-breast cancer cells in response to AA. This cascade was down regulated in ER- cells only. Cells were treated with AA at 6 and 24 hrs. RNA was isolated and hybridized on the cDNA microarray slides as detailed in materials and methods. Images were analyzed using GenePix 6.0 and data were analyzed using GeneSpring 10.1.
The peroxisome proliferator-activated receptor (PPAR) related pathways were significantly down regulated in ER+ cells by Arachidonic acid. These genes are listed in Table 7.

Five genes that were regulated were selected for real-time polymerase chain reaction (PCR). These genes are Protocadherin, thyroid hormone receptor-associated protein complex component (TRAP150), Mitochondrial ribosomal protein L43, transducer of ERBB2, WNT-2B Isoform 1 oncogene and coiled-coil domain containing 61 (CCDC61). Real-time PCR was carried out using samples from ER- cells (HCC-1806 and Hs578T) and ER+ cells (CAMA-1 and HCC-70) treated with the fatty acids and compared to the control untreated cells [Figure 8].

### DISCUSSION

Previous studies have emphasized that the correlation between postmenopausal breast cancer risk and dietary consumption is, for the most part, dependent upon the estrogen receptor status. Scientists have reported that the association between the Alternate Healthy Eating Index (AHEI), the Recommended Food Score (RFS) and the risk of breast cancer were found only in ER- tumors.[12]

Many studies focused on the effect of dietary intake of fatty acids and other nutrient on breast cancer. However, a detailed understanding of the correlation between dietary fat intakes and the ER status of breast cancer is not very well achieved.

A study on the association of alcohol intake and breast cancer risk showed no association between alcohol intake and the risk of developing ER- tumors while a statistically significant correlation between alcohol intake and the risk of developing ER+ tumors was observed.[13] McCann et al. have shown that the anti-tumor effects of dietary lignans, found in flaxseed, sesame seed and oat bran, are limited to ER- breast tumors.[14]

A study of the dietary intake of fatty acids in premenopausal breast cancer patients found an association between linoleic acid intake and a higher risk of ER- than ER+ breast tumors.[5] The omega-3 fatty acids, EPA and DHA, are shown to inhibit the growth of ER- and ER+ breast cancer cells in vitro.[15,16]

In this study, we determine whether the ER status of breast cancer cells plays a role in their responses to fatty acids at the molecular level, using microarrays. We have identified genes and pathways that are differentially expressed between ER- and ER+ cells in response to EPA and AA. The effect of EPA on cell-cell signaling was dependent on the ER status and included the activation of the caspase cascade in ER+ cells while the activation of the Cyclin-dependent kinase cascade was uniquely activated in ER- cells.

Functional interactions between ER and beta-catenin through transcriptional modulation is an important factor for in vivo cross-talk of beta-catenin and estrogen signaling pathways. Transcription coactivators and chromatin remodeling complexes that are normally recruited by beta-catenin are shown to interact with ER, and yet ER and beta-catenin are reciprocally recruited to cognate response elements in the promoters of their target genes. This interaction may underlie the pathological conditions in which abnormalities of beta-catenin signaling have been implicated.[17] In tumor
Figure 8: The gene expression data obtained by Real-time PCR experiment. ER- cells (HCC-1806 and Hs578T) and ER+ cells (CAMA-1 and HCC-70) were incubated with either AA (A.) or EPA (B.) for six hours. At the end of the incubation period, the cells were washed with PBS and TriZol was added. Total RNA was isolated analyzed using RT-PCR. Data points are the mean and standard error of three independent experiments for the same samples used in the microarray experiments. Gene expression data were normalized to GAPDH mRNA that showed no regulation among the various treatments compared to the untreated control cells.
cells, expression of ER down regulates beta-catenin and its target genes, cyclin D1 and Rb, important regulators of cell cycle and cell proliferation. Over expression of ER induces cellular apoptosis by inducing hTNF-alpha gene expression, which in turn activates caspases -8, -9 and -3 and lead to DNA fragmentation.[19]

One of the pathways that were differentially expressed in ER+ cells in response to AA was the ERK/MEK pathway.

For many years, the involvement of the ERK/MEK cascade in cell growth and the prevention of apoptosis have been investigated. Studies have shown that the ERK/MEK pathway can induce the progression of cancer cells due in part to the inhibition of apoptosis.[19-22]

It has been well documented that there is cross talk between the ER pathway and the ERK/MEK cascade and that the anti-apoptotic effect of estrogen may be partly dependent on the ERK/MEK pathway.[23, 24]

Arachidonic acid is shown to differentially induce the insulin signaling pathway in ER- cells. Genes involved in insulin receptor signaling pathway such as insulin like growth factors (IGF-I and –II) have been found to induce growth of many breast cancer cells. Expression of IGF-I receptor (IGF-IR) shown to be highly activated in breast tumors in comparison with normal epithelial cells.[25] Over expression of insulin receptor signaling genes, which aggravate proliferation of breast cancer cells, is worse in ER- patients. For example, ER- breast cancer patients have higher insulin like growth factor binding proteins levels than ER+ patients.[26] Elevated expression of IGF-IR or Insulin receptor substrate 1 (IRS-1) appears to increase drug- and radio-resistance of breast cancer cells and favor cancer recurrence.[27] Insulin receptor substrate 1 (IRS-1) is important in transmitting IGF-IR signals to counteract ER apoptotic effect through the PI-3K/Akt survival pathways, and its stabilization improved survival of breast cancer cells in the presence of IGF-I.[28]

Also documented is the cross-talk between the PPAR and ER pathways has been documented.[29,30] The PPAR cascade was uniquely down regulated in ER+ cells in response to arachidonic acid and not altered in ER- cells.

Our findings suggest that the ER status of breast cancer cells may play a role in breast cancer cell response to treatments with omega-3 and omega-6 fatty acids.

Further investigation of these pathways may shed light on the importance of the ER status on the mechanistic and therapeutic/preventive roles of fatty acids in breast cancer.

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