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ORIGINAL ARTICLE

Relationships of ESR1 and XBP1 expression in human breast carcinoma and stromal cells isolated by laser capture microdissection compared to intact breast cancer tissue

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Abstract Results from investigations of human genomics which utilize intact tissue biopsy specimens maybe compromised due to a host of uncontrolled variables including cellular heterogeneity of a sample collected under diverse conditions, then processed and stored using different protocols. To determine the cellular origin and assess relationships of mRNA expression of two genes reported to be co-expressed in human breast carcinoma (estrogen receptor-α, ESR1 and X-box binding protein 1, XBP1), gene expression analyses were performed with intact tissue sections and compared with those of laser capture microdissection (LCM)-procured carcinoma and stromal cells from serial sections of the same tissue. Frozen sections of human breast carcinomas were first evaluated for structural integrity and pathology after hematoxylin and eosin (H&E) staining. Total RNA preparations from intact tissue sections and LCM-procured carcinoma and stromal cells were reverse transcribed for measurements of ESR1 and XBP1 expression by quantitative PCR (qPCR). These results were compared with those obtained from microarray analyses of LCM-procured carcinoma cells. Levels of ESR1 and XBP1 were detected in the intact breast cancer tissue sections suggesting coordinate gene expression. Although coordinate expression of these genes was observed in the LCM-procured carcinoma cells, it was not discerned in LCM-procured stromal cells. The origin of coordinate expression of ESR1 and XBP1 observed in whole tissue sections of human breast cancer biopsies is due principally to their co-expression in carcinoma cells and not in the surrounding stromal cells as substantiated using LCM-procured cells. Collectively, a microgenomic process was established from human tissue preparation to RNA characterization and analysis to identify molecular signatures of specific cell types predicting clinical behavior.

Keywords Gene expression · ESR1 · XBP1 · Laser capture microdissection · Carcinoma cells · Stromal cells

Introduction

Gene expression profiles present a global view of cellular processes, which focuses efforts to better understand mechanisms of complicated diseases, as well as provides novel molecular targets for drug design. While certain studies utilized data sets obtained from human cell lines, e.g., [1–4], others directly employed biopsies of human tissues to reveal molecular profiles reflecting the status in vivo [5–8]. Prior to this elevated interest in molecular medicine, collection of human tissue biopsies focused primarily on their use in a clinical setting, with little emphasis on preservation for future genomic and proteomic analyses. Differences in tissue collection and handling, as well as the cellular heterogeneity of tissue sections complicated many investigations, often resulting in misleading findings when results were compared with those using individual cell populations, e.g., [9–12]. Laser capture microdissection (LCM) provides a non-destructive

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method for procuring homogeneous cell populations for molecular and biochemical analyses, such as gene expression studies, e.g., [13–16].

After specific populations of cells have been isolated, RNA maybe extracted for genomic analyses, such as quantitative PCR (qPCR) and microarray, e.g., [12, 16]. Many RNA isolation kits are commercially available; however, most require large amounts of starting material, which is impractical for LCM-associated protocols. This report describes a reproducible microgenomic process beginning with human tissue biopsy preparation at the time of frozen section collection to mRNA extraction, characterization, and analyses of gene expression in specific cell types to identify molecular signatures predicting clinical behavior.

The protein product of ESR1, estrogen receptor-α, is an established breast cancer biomarker, e.g., [17, 18], and is widely investigated in tissue biopsies and cells in culture. However, expression of ESR1 in the stromal cells surrounding carcinoma cells is less understood. An earlier investigation of breast tumor stromal and epithelial cell lines derived from human tissues indicated that the enzyme aromatase is present in stroma within breast tumors and suggests estrogen synthesis from within the tumor may modulate tumor growth by a paracrine mechanism [19]. Another study reported that estrogen was produced and released from the stroma surrounding human breast carcinoma cells [20]. However, ESR1 gene expression appeared to be similar in stromal cells microdissected from cancer biopsies when compared with cells from benign breast tissue [21].

X-box binding protein 1 (XBP1), an alternatively spliced transcription factor that is a member of the basic region/leucine zipper protein family, is involved in unfolded protein response, which prevents accumulation of unfolded and misfolded proteins in cells [22, 23]. Its over-expression appears related to altered expression of several apoptotic and cell cycle associated genes, promoting cell survival [22]. Over-expression and splicing of XBP1 were reported previously to be associated with poor outcome in breast cancer patients [24]. XBP1 appears to be a key factor in anti-estrogen responsiveness and estrogen dependence in breast cancer cells [22, 24], and its expression correlates with ESR1 in breast cancer cell lines [23, 25, 26]. Previous studies suggest XBP1 activates ERα in a ligand-independent manner [27, 28], and that the genes are co-expressed in breast cancer biopsies [29, 30]. To determine the cellular origin and assess relationships of mRNA expression of two genes reported to be associated in breast carcinoma (ESR1 and XBP1), gene expression analyses were performed with intact tissue sections and compared with those of LCM-procured carcinoma and stromal cells from the same tissue biopsy.

Materials and methods

Processing of human tissue specimens

To evaluate differences between cell types present in biopsies of invasive ductal carcinoma of the breast, either intact tissue specimens or cells isolated by LCM were extracted for gene expression analyses, e.g., [11, 12, 31–33]. Figure 1 illustrates the protocol for gene expression analyses of de-identified frozen tissue sections or of LCM-procured cells. All specimens and clinical follow-up information were de-identified and encoded in the Tumor Marker database as approved by the Institutional Review Board, and no identifiers (PHI) were used in this investigation. As previously described [12, 18, 31, 34], tissue specimens were processed and cryopreserved to retain the biological integrity of macromolecules [11, 12, 35]. Tissue specimens were frozen in TissueTek™ O.C.T. medium (VWR Scientific Products Corp., West Chester, PA), and stored at −80°C until sections were collected on uncharged slides for microdissection [11, 12, 35].

Prior to analyses, the structural integrity of the frozen tissue sections was evaluated after hematoxylin and eosin (H&E) staining (Fig. 2), using a modified protocol [12, 16, 31]. This modified protocol was used to shorten the time required, and thus reduce RNA degradation, while adequately staining the sections for visualization of cell types without a cover slip.

RNA isolation and characterization from intact tissue sections

Serial sections of frozen tissue biopsies were prepared as "curls," which were placed immediately into chilled tubes and extracted with 350 µl RLT buffer from the RNeasy® RNA isolation kit (Qiagen, Valencia, CA). The tissue was incubated on ice with brief (10 s) mixing using a Vortex Genie™, and was subjected to centrifugation at 800×*g* for 2 min to sediment the cell debris and O.C.T. embedding

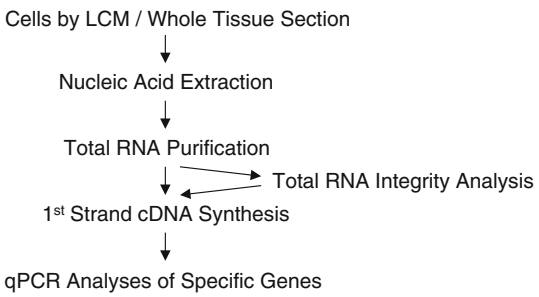

Fig. 1 Protocol for gene expression analysis of frozen tissue sections or of LCM-procured cells. Details of the procedures used to prepare tissue or cell specimens for the steps illustrated above are described in "Methods and materials" section

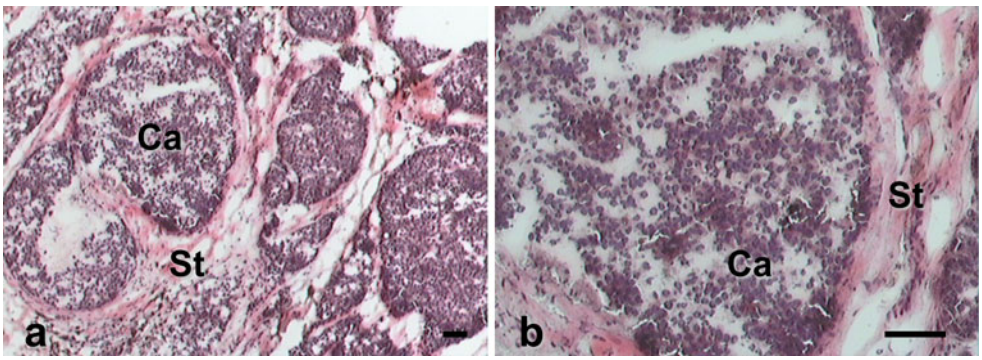

Fig. 2 Assessment of structural integrity. H&E staining of a tissue section from a representative breast cancer specimen that illustrates the preponderance of carcinoma cells (Ca) invading adjacent stroma (St). *Bar* = 40 μm

compound. Total RNA was isolated using the RNeasy® RNA isolation kit (Qiagen), including a DNase treatment step. Typically, 10–200 ng total RNA were isolated from a single 7 μm gross tissue section, e.g., [16]. If only a small amount of RNA remained intact in this assessment of sample quality (e.g., less than 10 ng for downstream qPCR analyses), then subsequent LCM procedures were not warranted. Quality of RNA was evaluated with the Agilent RNA 6000 Nano Kit and the Bioanalyzer™ (Agilent Technologies, Palo Alto, CA).

RNA isolation and characterization from LCM-procured cells

Cells of interest were microdissected using the PixCell IIe™ with CapSure™ LCM Caps (Molecular Devices, Sunnyvale, CA), which permitted collection of intact cells on the surface transfer film of the cap (Fig. 3). Typically, cells were collected using 3,000 pulses (7.5 μm "spot" size) of the laser within 30 min of H&E staining. Total RNA from LCM cells was extracted using the PicoPure® RNA Isolation kit (Molecular Devices) with a DNase (Qiagen) digestion, which is optimized for the small numbers of cells procured by LCM. Extracted RNA was evaluated with the Agilent RNA 6000 Pico Kits and the Bioanalyzer™ (Agilent Technologies). Typically, 1–6 ng of total RNA were extracted from LCM-procured cells, which was similar to earlier studies [11, 12, 16, 31].

First strand synthesis and qPCR analyses of gene expression

Total RNA from either the intact tissue section or LCM-procured cells was reverse transcribed in a solution of 250 mM Tris–HCl buffer, pH 8.3 containing 375 mM KCl, and 15 mM $MgCl_2$ (Invitrogen), 0.1 M DTT (dithiothreitol, Invitrogen), 10 mM dNTPs (Invitrogen), 20 U/reaction of RNasin™ ribonuclease inhibitor (Promega, Madison, WI), and 200 U/reaction of Superscript™ III RT (reverse transcriptase, Invitrogen) with 5 ng T7 (oligo (dT))

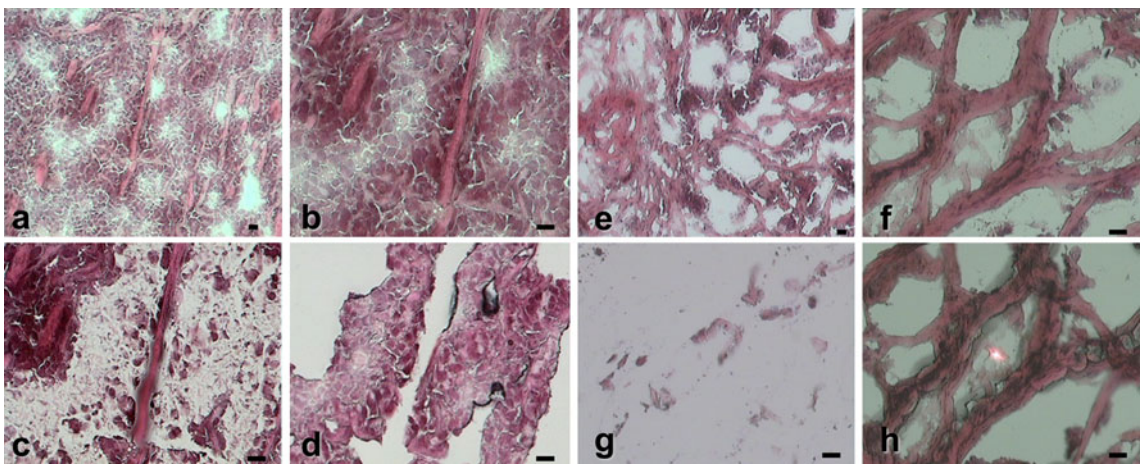

Fig. 3 Images from LCM performed on a representative H&E stained invasive ductal carcinoma tissue section. A map image is taken at ×10 magnification (**a**, **e**), while LCM is performed at ×20 magnification (**b**–**d**, **f**–**h**). Image **c** illustrates the complete removal of carcinoma cells by LCM, which have been deposited on the surface of the LCM cap (**d**, **h**). *Bar* = 20 μm

Table 1 Primer sequences utilized in this study

Gene ID	Forward primer	Reverse primer
ACTB	AACTGGTCTCAAGTCAGTGTACAGG	TCCCCCAACTTGAGATGTATGAAG
ESR1	GCCAAATTGTGTTTGATGGATTAA	GACAAAACCGAGTCACATCAGTAATAG
XBP1	CCCCCTTTTTGGCATCCT	GCAGGTGTTCCCGTTGCTTA

primers. The cDNA obtained from this reverse transcription reaction was diluted tenfold in 2 ng/μl polyinosinic acid for use in qPCR reactions.

qPCR reactions were performed in a total volume of 10 μl/well containing diluted cDNA, Power Sybr™ Green PCR Master Mix (Applied Biosystems, Foster City, CA), forward and reverse primers. Primers were designed with Primer Express™ (Applied Biosystems) to generate sequences closer to the 3′ end of the transcript for use with the T7 primers in the reverse transcription (Table 1). qPCR reactions were performed in triplicate with duplicate wells in each 384-well plate. Gene expression was normalized to *β*-actin (ACTB) and compared with Universal Human Reference RNA (Stratagene, La Jolla, CA), to calculate a relative expression level [36].

Microarray analysis of gene expression

Data from previous microarray studies [37–39] of LCM-procured carcinoma cells from 247 frozen patient samples were utilized for this investigation. Pearson correlations were performed in IBM® SPSS® Statistics 19. Gene relationships and pathway analyses were performed in Ingenuity® IPA® 8.5.

Table 2 Representative results showing the quantity of total RNA extracted under different conditions using tissue sections from a de-identified breast cancer specimen

Sample ID	RNA extracted (ng)	Recovery (%) compared to unstained section
1A: unstained	19.7	
1B: H&E stained	12.3	62.4
1C: LCM cap (2,221 laser pulses)	5.6	28.4
2A: unstained	7.4	
2B: H&E stained	5.0	67.6
2C: LCM cap (2,137 laser pulses)	3.3	44.6
3A: unstained	14.5	
3B: H&E stained	6.0	41.4
3C: LCM cap (1,604 laser pulses)	2.5	17.2

Serial sections of representative specimens of invasive ductal carcinoma of the breast were prepared and a section was unstained (A), while a second serial section was stained with H&E (B). The third serial section, stained with H&E (C), was subjected to LCM for procurement of cancer cells only

Results

Intact tissue section analyses

Analysis of the intact tissue section is vital to ensure high-quality RNA of sufficient quantity before proceeding with the LCM process. Tissue was stained by H&E with a protocol identical to that employed for tissue sections used for LCM to ensure there was no difference attributable to the staining in the extent of RNA degradation in the preparation of each sample, i.e., intact tissue section and microdissected cells. As illustrated in the H&E stained tissue section from a representative breast carcinoma specimen (Fig. 2), the structural integrity indicates it was acceptable to proceed with gene expression analyses.

Assessment of RNA yield and integrity

In preparation for genomics studies utilizing LCM-procured cells, RNA yield and integrity analyses of the cognate intact tissue section were performed. To assess RNA obtained from either tissue sections or LCM-procured cells, serial sections of each of three specimens, representative of the invasive ductal carcinomas used in this study, were prepared and one section was maintained unstained, while another was stained with H&E (Table 2). The third section was subjected to LCM of carcinoma cells only. The results shown in Table 2 are representative of differences observed between RNA quantities extracted from H&E stained sections compared with unstained sections. As predicted, the quantity of total RNA in the LCM-procured cell preparation varied with the number of cells captured.

Assessment of yield and integrity of RNA from LCM-procured cells

Homogeneous populations of stromal and carcinoma cells were procured from individual specimens to generate gene expression results from each cell type to compare the relative levels within a single carcinoma biopsy. To examine gene expression independently in carcinoma and stromal cells from a single breast cancer biopsy, frozen tissue blocks were processed as serial 7 μm sections (Fig. 1), and at least 1,000 breast carcinoma cells or 1,000–2,000 breast stromal cells were procured by LCM for RNA extraction

Table 3 Representative quantities of RNA extracted from LCM-procured cells

Sample	Number of LCM caps	Total number of laser pulses	[RNA] (ng/ul)	Total yield of RNA (ng)	Yield of RNA (pg)/pulse
1: Cancer cells	2	7,730	3.7	44.4	5.7
1: Stromal cells	2	8,282	1.3	15.6	1.9
2: Cancer cells	2	12,824	7.1	85.2	6.6
2: Stromal cells	2	7,522	4.7	56.4	7.5
3: Cancer cells	2	4,790	4.2	50.4	10.5
3: Stromal cells	2	2,024	1.8	21.6	10.7
4: Cancer cells	3	9,565	7.2	86.4	9.0
4: Stromal cells	3	5,042	2.7	32.4	6.4
5: Cancer cells	2	7,779	10.1	121.2	15.6
5: Stromal cells	2	5,265	5.6	67.2	12.8
6: Cancer cells	1	8,250	3.8	45.6	5.5
6: Stromal cells	1	4,230	1.5	18.0	4.3
7: Cancer cells	2	8,378	13.6	163.2	19.5
7: Stromal cells	1	5,562	9.8	117.6	21.1

and analyses. Images from LCM performed on a representative invasive ductal carcinoma are shown in Fig. 3. For documentation purposes, an image is taken at ×10 magnification (3a and e), and LCM is performed at ×20 magnification (3b–d, f–h). Image c illustrates the removal of carcinoma (Fig. 3c) or stromal cells (Fig. 3g) by LCM, which have been deposited on the surface of the LCM cap (Fig. 3d, h). Multiple cell captures were performed on many samples, and RNA was pooled to obtain sufficient quantities for qPCR reactions (Table 3). As shown in Table 3, the yield of RNA per laser pulse was similar for carcinoma and stromal cells, regardless of the number of pulses used in a single tissue section. Bioanalyzer™ analyses confirmed the integrity of the extracted RNA, although the RNA profiles from some stromal cell extracts indicated increased amounts of RNA species with molecular weights lower that 18S rRNA. It is unclear if these low molecular weight RNA species are related to the presence of native RNA molecules or simply to RNA degradation during LCM collection.

Gene expression analyses by qPCR and microarray

In order to assess ACTB as a potential reference gene in this investigation, the following study was performed. Each of eight RNA samples of a breast tissue panel was diluted to the same concentration and re-quantified by spectroscopy (Nanodrop™) to confirm the concentrations. The RNA was reverse transcribed and subjected to qPCR in duplicate for the reference gene, ACTB (Table 4). Results from these eight samples gave an average Ct value of 18.58 with a standard deviation of 0.54, indicating a relatively low amount of variation of ACTB expression among samples. Thus, ACTB was employed as the reference gene in the standardized protocol for breast tissue evaluation by LCM. Standard curves of ESR1, XBP1, and ACTB were developed using dilutions of cDNA prepared from Universal Human Reference RNA (Stratagene/Agilent, La Jolla, CA) indicating similar amplification efficiencies (i.e., slope of ACTB ± 0.1), which is vital for normalization of gene expression [40]. Dissociation curve analyses confirmed a single PCR product in the qPCR reactions.

Table 4 Representative results evaluating ACTB as a normalizing gene for use in gene expression studies of human tissue

Sample	Average C_t value
1	18.90
2	18.05
3	17.99
4	19.01
5	18.73
6	19.39
7	17.92
8	18.65
Average C_t value	18.58
Standard deviation	0.54

Extensive quality control experiments were performed to assess reproducibility of the qPCR results. Four serial tissue sections from each of three specimens were prepared, RNA was isolated, reverse transcribed, and qPCR analyses were performed for expression of ESR1 (Fig. 4). An ANOVA test (Kruskal–Wallis) was performed on the gene expression results for ESR1, and no statistically significant difference was observed [41]. The coefficient of variation (CV) was calculated for ESR1 expression in each

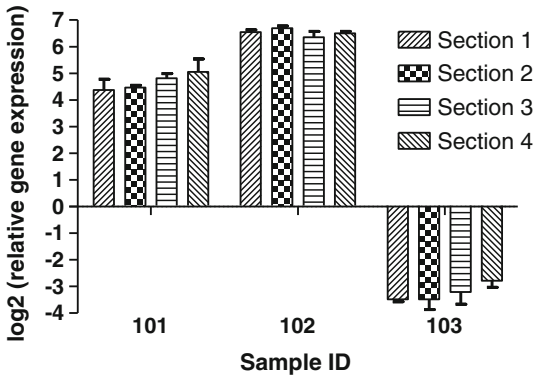

Fig. 4 Reproducibility of ESR1 gene expression measurements in four serial tissue sections of three carcinoma specimens

specimen to identify the relative variability. CV values of ESR1 measurements in four serial sections from each of three specimens were 13.5, 30.5, and 37.2%. Using criteria from previous reports examining gene expression results [42–44], measurements with less than 50% CV represent acceptable levels of relative variability.

The procedures described were employed to investigate the expression of XBP1 and ESR1 in frozen intact tissue sections. In addition, LCM was utilized to isolated specific carcinoma and stromal cell populations from serial tissue sections, and expression levels of ESR1 were determined (Fig. 5a). There were significant differences in ESR1 gene expression levels between intact tissue and LCM-procured carcinoma cells in only two of the 13 specimens evaluated (samples 12 and 13). Six specimens (samples 1, 4, 7, 9, 12, and 13) exhibited differences in levels of ESR1 gene expression in stromal cells compared to those of the intact tissue. Seven biopsies (samples 1, 2, 4, 7, 8, 12 and 13) exhibited differences in ESR1 gene expression when comparing the LCM-procured carcinoma and stromal cells. Six of these samples exhibited decreased expression in the stromal cells compared with levels in the carcinoma cells. Nonetheless, results indicate that ESR1 mRNA is present in stromal cells of certain invasive breast carcinomas as previously suggested [21, 45–47], implying a role for estrogen in the tumor microenvironment.

To investigate the cellular origins of XBP1 expression, LCM-procured carcinoma and stromal cells were utilized in addition to the gene expression from the entire tissue section (Fig. 5b). XBP1 gene expression levels were significantly different in six preparations of the LCM-procured carcinoma cells compared with that of the intact tissue (samples 1, 3, 6, 9, 11, and 12). Furthermore, differences in XBP1 gene expression in LCM-procured stromal cells compared with that of the intact tissue were detected in ten of 13 specimens. When XBP1 mRNA levels were compared in LCM-procured carcinoma and stromal cells, nine of the 13 specimens exhibited differences. Seven of these samples exhibited decreased expression in the stromal cells compared with the carcinoma cells. Collectively the data suggest that XBP1 gene expression is greater in breast carcinoma cells compared with the adjacent stromal cells.

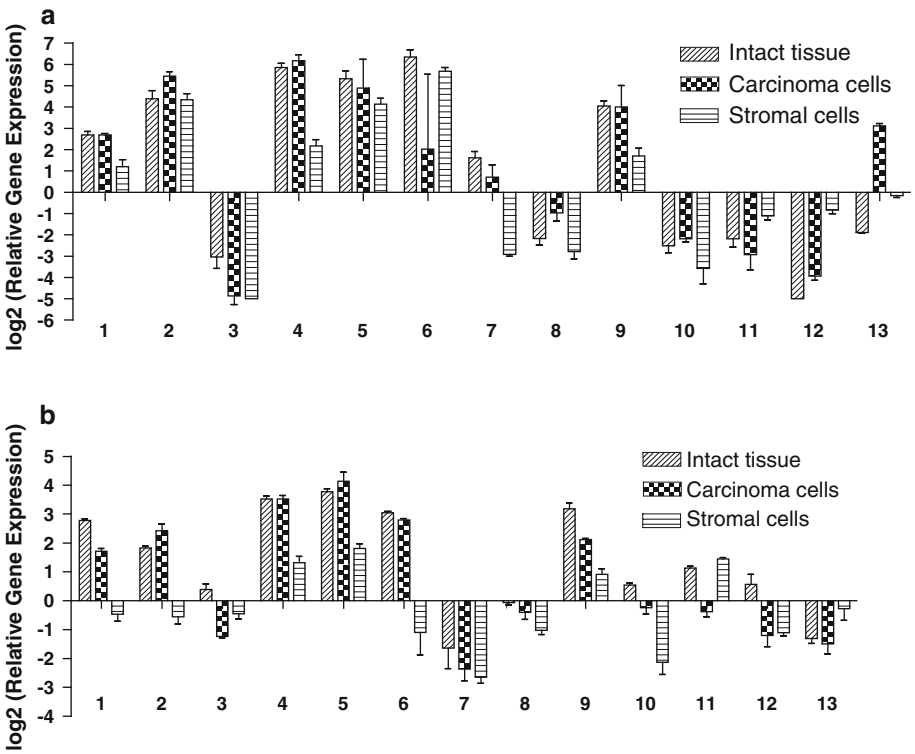

Fig. 5 Thirteen representative breast carcinoma biopsies analyzed for expression of ESR1 (**a**) and XBP1 (**b**). Gene expression was measured in the intact tissue section and in LCM-procured carcinoma and stromal cells

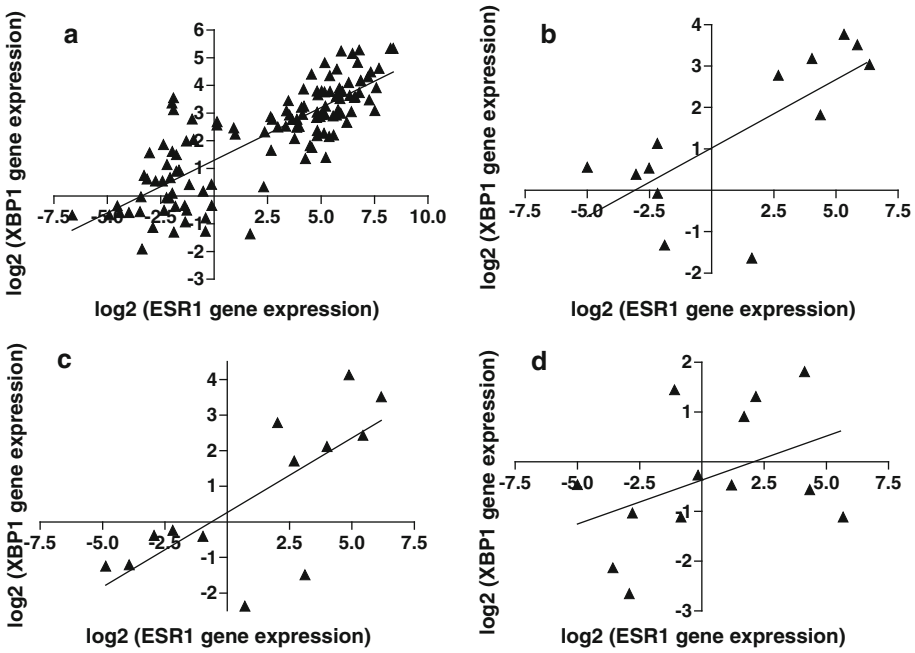

Fig. 6 Correlations of ESR1 and XBP1 gene expression measured by qPCR. Comparisons of ESR1 and XBP1 are shown in 126 intact tissue sections of invasive ductal carcinoma of the breast (**a**). A similar correlation is shown in the 13 intact tissue specimens also investigated by LCM (**b**). Correlation of ESR1 and XBP1 gene expression in carcinoma cells isolated by LCM (**c**), and in LCM-procured stromal cells (**d**) is shown

To ascertain the relationships between coordinate expression of ESR1 and XBP1 genes in the specific cell types and in whole tissue sections, qPCR results were correlated for each. Pearson correlations indicated a significant correlation (0.82, $P < 0.001$) between ESR1 and XBP1 expression levels in 126 intact tissue sections examined (Fig. 6a), which is consistent with the correlation of 0.72 ($P = 0.005$) observed in the 13 intact tissue sections which were subjected to LCM procurement of specific cell types (Fig. 6b). Interestingly, a significant correlation (0.73, $P = 0.004$) was observed between ESR1 and XBP1 expression levels in the LCM-procured carcinoma cells (Fig. 6c), which was not observed (0.43, $P = 0.14$) when ESR1 and XBP1 expression was compared in the LCM-procured stromal cells (Fig. 6d).

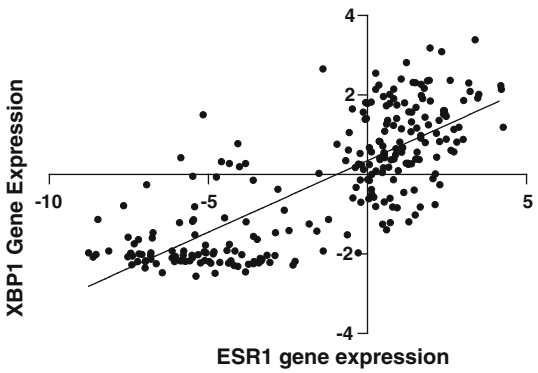

Fig. 7 Correlations of ESR1 and XBP1 gene expression in carcinoma cells measured by microarray. Comparisons of ESR1 and XBP1 are shown in in LCM-procured carcinoma cells of 247 breast cancer patient specimens

Further investigation of the relationship of ESR1 and XBP1 in carcinoma cells was performed utilizing microarray data obtained from LCM-procured carcinoma cells from 247 breast cancer patients. Pearson correlations showed a significant relationship (0.78, $P < 0.001$) between the two genes (Fig. 7), confirming the relationship shown in the 13 patient set analyzed by qPCR.

Discussion

In this investigation, procedures were refined [11, 12] for processing human tissue biopsies for microgenomic-based investigations, including RNA purification and amplification from both frozen tissue sections and LCM-procured cells. It was demonstrated that the total RNA extracted from either frozen tissue sections or from individual cell populations (e.g., carcinoma or stromal cells) collected by LCM was of high quality for gene expression analyses. Furthermore, standardized conditions were developed to improve RNA yields from LCM-procured cells, as well as from intact tissue sections, such that qPCR could be performed reproducibly.

The choice of reference gene is vitally important for normalizing qPCR data. The gene chosen must be evenly expressed across samples and amplify with the same efficiency as the genes of interest, to ensure that differences observed reflect the biological status of the specimen. Although a study [48] reported that greater than 90% of gene expression measurements published in high impact journals before 1999 utilized either GAPD, ACTB, 18S or 28S rRNA as single genes for normalization, other

investigators question whether any single gene is ideal (e.g., [49, 50]). The latter authors' suggestions included the use of total RNA or panels of reference genes. Although these studies focused on identification of genes whose expression levels remained constant in a variety of cell types, use of a single tissue or cell type suggests the reference gene must remain constant only in that particular tissue (e.g., [50]). This maybe confirmed by analyses of several tissue samples, each with known RNA concentrations, as suggested previously by Suzuki et al. [48]. Results from our laboratory demonstrated that ACTB was a valid gene for normalization of qPCR results, since its expression levels remained constant among a variety of human breast carcinomas exhibiting considerable cellular heterogeneity (Table 4), and its amplification efficiency was similar to those of target genes. Collectively, these results confirm that the procedures for processing intact tissue and populations of specific cell types for subsequent isolation of intact mRNA were applicable for assessing the expression of ESR1 and XBP1.

ESR1 and XBP1 gene expression levels were correlated in 126 intact breast carcinoma tissues in a relationship similar to that reported previously using breast cancer cell lines [22, 23]. This coordinate expression appears to be related to previous findings that XBP1 plays a role in the ligand-independent activation of ERα leading to estrogen independent growth of breast cancer cells in culture [22, 27, 28]. The more aggressive behavior of breast carcinomas over-expressing XBP1 is apparently related to their resistance to anti-estrogen treatment. Over-expression of XBP1 also increased the cell's unfolded protein response, which has been shown to decrease a tumor's sensitivity to chemotherapeutics [51].

Expression of ESR1 and XBP1 appears to be coordinate in both intact cancer tissue sections and in LCM-procured carcinoma cells implying that the consequence of these gene products is more profound in the carcinoma cells themselves, compared with their effects in surrounding stroma. This is supported by our observation that ESR1 and XBP1 gene expression was significantly lower in populations of stromal cells isolated by LCM from the same tissue specimen. A study by Myhre et al. [52] reported gene expression differences primarily in carcinoma cells but not in the surrounding stroma of breast cancer patients with and without distant metastases; however, this investigation was performed in silico and not in LCM-procured populations of carcinoma and stromal cells, as reported in our study.

Using Ingenuity® IPA® 8.5 to analyze pathway interactions, both ESR1 and XBP1 appeared in a network highly associated with cellular development, growth, proliferation, and the cell cycle. This network also includes genes with known relevance in cancer behavior, such as BCL2, ERBB2, and MYC. Two of these, BCL2 and ERBB2, appear in a gene signature we have identified that predicts risk of recurrence of early stage, estrogen receptor positive breast carcinoma [Kerr and Wittliff, accepted for publication].

Increased expression of ESR1 mRNA is related to elevated levels of estrogen receptor protein in breast carcinoma cells, which is generally correlated with better prognosis [18, 34, 53]. However, not all patients with estrogen receptor positive breast cancer exhibit decreased disease-free survival [18]. Furthermore, when XBP1 expression in cancer tissue biopsies is considered as an independent variable, it is reported to be correlated with clinical outcome [24]. Collectively, our results showing coordinate expression of ESR1 and XBP1 mRNA in LCM-procured breast carcinoma cells compared with surrounding stroma, suggest these genes are closely associated with the molecular mechanisms of aggressive growth behavior of certain cancers exhibiting ESR1.

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Conflicts of interest The authors declare that they have no conflict of interest.

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