A Novel Putative Low-Affinity Insulin-like Growth Factor-binding Protein, LIBC (Lost in Inflammatory Breast Cancer), and RhoC GTPase Correlate with the Inflammatory Breast Cancer Phenotype

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INTRODUCTION

The term IBC was first coined in 1924 by Lee and Tannenbaum (1) to describe a form of locally advanced breast cancer characterized by erythema, skin nodules, pain, changes in range, and nipple retraction. Although all forms of locally advanced breast cancer can be associated with skin nodules and erythema, IBC is clinically distinguished by the rapid onset of these changes, typically arising within 6 months, and is diagnosed as stage IIIb (2, 3). Primary IBC accounts for ~6% of new breast cancer cases in the United States annually. With a mean 5-year disease-free survival rate of <45%, IBC is the most lethal form of locally advanced breast cancer (4). Despite the often-morbid outcome of the disease and the fact that IBC is well characterized clinically (2), very little is known about the genetic alterations involved in the etiology and progression of this disease. IBC has lower rates of expression of estrogen and progesterone receptors and faster growth kinetics than non-IBC (5). Key genes known to be involved in carcinogenesis such as p53, c-myc, p52, and c-erbB2 (6–8) have been shown to have varied levels of expression in IBC. However, no alteration in any of these genes has emerged as specifically characteristic of the inflammatory phenotype. The rapid progression of IBC is not associated with precursor lesions and, from the outset, is highly invasive, especially in the skin. We hypothesized that a limited number of genetic alterations give rise to this constellation of distinct clinical characteristics.

In this study, we compared the differential expression of transcripts from a primary IBC cell line (SUM149) with actively growing normal mammary epithelial cells and the patients’ matched lymphocytes. Seventeen genes, each expressed by the normal cell lines (and not by the tumor) and nine over-expressed by the tumor cell line, have been identified. This is the first study of the identification and characterization of genes that play a role in determining the phenotype of IBC.

MATERIALS AND METHODS

Cell Lines. Cell lines were maintained under defined, well-tailored culture conditions optimal for growth in each case (9, 10). The SUM149 cell line was established from a primary IBC and grown in 5% FBS (Sigma Chemical Co., St. Louis, Missouri).
MO) supplemented with Ham’s F12 medium (JRH Biosciences, Lenexa, KS) containing insulin and hydrocortisone (Sigma Chemical Co.). Lymphocytes of the SUM149 patient were immortalized with EBV, as described (11, 12). Lymphocytes were grown in 10% FBS supplemented with RPMI medium (Life Technologies, Inc., Gaithersburg, MD). Normal HME cells were immortalized with human papilloma virus (13) and grown in 5% FBS supplemented with Ham’s F12 medium containing insulin, hydrocortisone, epidermal growth factor, and cholera toxin (Sigma Chemical Co.). The HME cells were shown to be keratin 19 positive and, therefore, of the same differentiation lineage as the SUM149 cell line. MCF10A cells were grown in Ham’s F12 medium supplemented with insulin, hydrocortisone, and epidermal growth factor. The remaining SUM breast cancer cell lines were cultured as described previously (9, 10). All cells were grown at 37°C in 10% CO2.

**Differential Display.** Differential display of mRNA was performed using a modified version of the method of Liang and Pardee (14, 15), with select components from the RNAimage kit (GenHunter Co., Nashville, TN). mRNA was isolated from cells that had reached 75% confluence using the Micro-FastTrack mRNA isolation kit (Invitrogen, Carlsbad, CA). The mRNAs were then reverse transcribed using a single-base anchored oligo-dT primer (H-T11-G, H-T11-C or H-T11-A). The first-strand cDNAs were then PCR-amplified using one of three primers of arbitrary sequence (H-AP1, H-AP2 or H-AP3) and a 32P-labeled oligo-dT primer. The PCR products were separated on a 6% denaturing polyacrylamide-sequencing gel and visualized on Hyperfilm (Amersham Life Sciences, Buckinghamshire, England). Each experiment was performed three separate times, starting from the mRNA isolation step to confirm differentially expressed transcripts between tumor and normal cells. Transcripts that were differentially expressed in the triplicate experiments were excised from the gel and reamplified with the original primer set used in the differential display step.

**Cloning and Sequencing.** Reamplified cDNA fragments were cloned using the pGEM-T Easy kit (Promega, Madison, WI). Plasmid DNAs were purified using the Wizard SV miniprep kit (Promega) and sequenced.

**5’ RACE.** RACE was performed by using the 5’ RACE kit (Life Technologies, Inc.). A gene-specific primer for the 3’ end of each differentially expressed transcript was used to generate cDNA from each mRNA isolated from either the SUM149 or HME cell lines. A poly(C) cap was attached to the 3’ end of the cDNA after elimination of remnant mRNA. An abridged anchored primer and a second gene-specific primer was used to amplify the cDNA. The PCR products were cloned into pGEM-T Easy and sequenced.

**Database Analysis.** Transcript sequences were compared with published gene sequences and expressed sequence tags by the BLASTN, dbEST, and TIGR (The Human Gene Index) databases. The protein sequences were aligned with the Megalign option of the Lasergene program.

**Northern Blot Analysis.** Total RNA (10 μg) from SUM breast cancer cell lines, MCF10A, and normal HME cells were isolated using Trizol reagent (Life Technologies, Inc.) and separated on a 1% formaldehyde agarose gel. The separated RNAs were transferred to a Nytran membrane using a TurboBlotter apparatus (Schleicher and Schuell, Keene, NH). Probes for each of the differentially expressed transcripts were cloned from the PCR product derived in the differential display step. Transcript-specific and glyceraldehyde -3-phosphate dehydrogenase (internal control) probes were 32P-labeled, and membranes were hybridized using the method of Church and Gilbert (16). Northern blots were visualized on X-OMAT film (Kodak, Rochester, NY).

**Radiation Hybrid Panel Mapping.** The Stanford G3 panel was used to determine the chromosomal locations of previously unmapped or novel genes. For each of the unmapped genes, PCR primers that produce specific 150–300-bp products from human genomic DNA with no cross-reactivity to rodent genomic DNA were generated. These primers were then used in a PCR reaction with the G3 panel, and each of the clones scored as positive, negative, or ambiguous. These PCR results were submitted to the WICGR mapping program to determine relative distance to chromosomal markers.

**Specimen Selection.** Archival samples were selected from stage IIIA and stage IIIB cases logged into a clinical breast cancer database associated with a clinical trial for locally advanced breast cancer (17). For each case, a thorough review of the clinical record was undertaken. The diagnosis of IBC was determined clinically when the record demonstrated that the patient had experienced a rapid onset (within 4 months of presentation) of grave signs of locally advanced disease, erythema, skin nodules, peau d' aurange, and/or nipple retraction. In some cases, the histological finding of tumor emboli in the ductal lymphatics was also seen. However, this feature, in the context of other typical clinical presentations, is not mandatory for a diagnosis of IBC.
absence of the clinical syndrome, was not sufficient to determine the inflammatory phenotype in our series. Unselected slowly progressing stage IIIA and noninflammatory stage IIIB breast cancers of all types were used as controls for the in situ hybridization experiments.

**In Situ Hybridization.** In situ hybridization was performed on archival paraffin sections, as described previously (18, 19, 20). Antisense 30-mer oligo DNA probes complementary for the differentially expressed mRNAs were designed from the Northern probe sequences. The probe sequences were submitted to the BLASTN database to ensure specificity. Probes were generated with 6-biotin molecules (Biotage Tail) on their 3’ end (Research Genetics, Huntsville, AL). The lyophilized probes were reconstituted to a final concentration of 1 g/ml in probe diluent (Research Genetics). Tissue sections were mounted on silane-treated ProbeOn slides (Fisher Scientific, Pittsburgh, PA). Slides were placed in the microprobe slide holder, dewaxed, and rehydrated with Autodewaxer and Autowax (Research Genetics). A probe aliquot was denatured at 95°C, 3 min, 30 sec, then cooled to room temperature. A poly d(T)20 probe was used to verify the integrity of the mRNA in each sample. The appropriate positive and negative controls were used for each case and probe. As a positive experimental control, each experiment included a section of A431 tumor, which overexpressed the EGF-receptor, probed with an EGF-R antisense oligo. Samples were analyzed in a Zeiss photomicroscope (Carl Zeiss, Thornwood, NY), the frequency and intensity of staining relative to the positive control was determined independently by two of the investigators who were blind to the sample classification. H&E staining was used to identify tumor, normal mammary epithelium, and other tissues.

**PCR-based Library Screening.** DNA (~1 ng) from an λZAP (Stratagene, La Jolla, CA) oligo-dT human ovarian cDNA library was amplified using different combinations of PCR primers (10 ng/μl) specific for the N8 transcript with the forward and reverse λZAP primers (10 ng/μl). Aliquots of the PCR products were separated on a 1.5% TAE agarose gel and excised. The PCR products were cloned into pGEM-T Easy and sequenced.

### RESULTS

**Isolation of Differentially Expressed Transcripts.** A modification of the differential display technique of Liang and Pardee (14, 15) was used to compare a primary IBC cell line, SUM149, with the patient’s own lymphocytes and two normal HME cell lines. Immortalized normal mammary epithelial cells were used for reference to obtain mRNA from a well-characterized, reproducible, actively replicating culture. These cells are keratin 19 (K19) positive, therefore, ensuring that the normal cells are from the same differentiation lineage as the inflammatory tumor cell line. mRNA transcripts were amplified by combinatorial pairings of single-base anchored oligo-dT primers with arbitrary forward random primers. Because differential display is known to give rise to false positive (spurious amplification) and false negative results, each experiment was performed under identical conditions three separate times, starting from the mRNA isolation step. Only those transcripts that were expressed in all three normal cell lines and not the SUM149 cell line, or expressed solely by SUM149 and not the normal mammary epithelial cell lines or lymphocytes, were considered for further characterization. This way, only those transcripts unique to normal cells and not due to human papilloma virus or EBV immortalization or individual polymorphisms can be identified. Fig. 1 illustrates the patterns of expression of the four selected cell lines using one of the primer sets. In this example, two transcripts (N1 and T1) are depicted; these represent transcripts expressed exclusively by the normal and tumor cell lines, re-
pectively. Using this technique, the gel patterns reveal both gains and losses of gene expression, allowing for the identification of both potential oncogenes and tumor suppressor genes. Overall, eight differentially expressed transcripts were repeatedly observed to be expressed exclusively in the normal cell lines, whereas nine transcripts were found to be expressed solely in the SUM149 IBC cell line (Table 1).

Identification of Transcripts Differentially Expressed between Normal Mammary Epithelial Cells and the SUM149 IBC Cell Line. The sequences of the transcripts isolated by the differential display technique generally correspond to the 3’ ends of the genes. Additional sequence for each transcript was determined by 5’-RACE and PCR-based library screening. All transcripts were further identified and characterized, except for the olfactory-receptor gene family members. In this case, efficient primers could not be synthesized to acquire larger pieces of the isolated transcripts. This was possibly due to the fact that each of olfactory-receptor gene fragments contained a conserved motif with about 10 unique bases in each transcript. These genes are known to be highly homologous and widespread throughout the human genome (21).

Sequences corresponding to the 5’ and 3’ ends of each of the transcripts isolated by differential display were submitted to three different databases: BLASTN, dbEST, and TIGR, then compared with previously published sequences. The results of database analyses are shown in Table 1. The putative gene EST or THC sequence is given along with the corresponding database used in the identification. We identified several known genes and published ESTs. Two transcripts correspond to novel genes with no database matches. The N5 and N6 transcripts were found to be alternatively spliced versions of the same gene and matched to the same EST.

Validation of Differentially Expressed Genes and Transcripts. To confirm that each of the transcripts was differentially expressed, and to compare levels of expression for each transcript across a broad range of breast cancers, Northern blot analysis was performed on a panel of low-passage cell lines representative of various types and stages of breast cancers. Total RNA was isolated from the 6-15 HME cell line, the MCF10A spontaneously immortalized breast epithelial cell line and 11 breast cancer cell lines, which included SUM149 and one other primary IBC cell line, SUM190 (Fig. 2A). Comparison of SUM149 with the 6-15 HME cell line confirmed differential expression of each transcript. In the case of the T6 transcript (RhoC GTPase), expression was seen in both the SUM149 cell line and the 6-15 HME cell line. However, the tumor cell line had an 8-fold increase in expression over the normal HME cell line (Fig. 2B). Southern blot analysis demonstrated that this increase in expression is not due to a detectable increase in gene copy number (Fig. 2C). Expression of each of the transcripts was seen to be variable across the panel of breast cancer cell lines, with no discernible trends observed. Similar trends of expression were seen in the SUM149 and the SUM190 IBC cell lines (data not shown).

The chromosomal location of each of the unknown genes was determined by radiation hybrid panel mapping. The results of these experiments, in addition to the previously reported chromosomal locations of some of the known genes are given in Table 1. The N5/N6 transcript, which has an EST match, localized to both chromosomes 7 and 9. This suggests a possible gene family for this transcript found on multiple chromosomes. The N8 transcript, which had an EST match located in PAC clone 142L7 (GenBank accession #HS142L7), was mapped to chromosome 6q21 independently by the chromosome 6 group at the Sanger Center. This transcript is located near marker D6S146. The N2 transcript, which matched to the KIAA0180 gene, mapped near chromosomal marker WI-1163. The T5 transcript corresponding to the overexpressed breast tumor protein mapped near marker WI-4822. Finally, the novel N9 and T8 genes were mapped near markers CHCC.GATA91E12 and CHCC.GATA45B10, respectively.

In Situ Hybridization of Archival Breast Cancer Specimens. To discern which of the genes identified and characterized in this study were specific to IBC and not to non-IBCs of the same stage, we probed 29 inflammatory and 19 noninflammatory stage III archival breast cancer samples by in situ hy-

![Fig. 2 A](#). Northern blot analysis of five key differentially expressed genes performed on a panel of breast cancer cell lines representing a range of breast cancer types and stages. Differential expression between the 6-15 HME cell line and the SUM149 IBC cell line was confirmed by this method. No discernable trends of alterations were apparent for the noninflammatory cell lines. The novel gene LIBC had only one band present at 2.5 kb. All transcripts identified by differential display were analyzed in an identical fashion by Northern blot. B, comparison of RhoC GTPase expression in the 6-15 HME cell line and the SUM149 IBC cell line by Northern blot analysis. The SUM149 IBC cell line demonstrated an 8-fold increase in RhoC GTPase expression when compared with the 6-15 HME cell line. C, Southern blot analysis of the SUM149 cell line and HME cell lines (6-5 and 6-15 HME) hybridized with a 900-bp probe for RhoC GTPase. Although RhoC expression was increased 8-fold in SUM149 over normal mammary epithelial cells, Southern analysis does not show gene amplification.
Percentage of archival inflammatory and noninflammatory breast tumors expressing each of the transcripts, as determined by in situ hybridization. The differential expression of two transcripts, LIBC (N8) and RhoC (T6), was statistically significantly altered in inflammatory tumors versus noninflammatory stage III breast cancers. Expression LIBC was lost in 80% of inflammatory tumors and 21% of noninflammatory tumors. RhoC was overexpressed in 90% of inflammatory tumors, whereas only in 38% of noninflammatory tumors. Both of these genes were concordantly altered in 91% of the tumors analyzed.

<table>
<thead>
<tr>
<th>Transcript</th>
<th>Identification</th>
<th>IBC (n = 29)</th>
<th>Non-IBC (n = 19)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcripts expressed exclusively by normal HME cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>H-NUC/cdc27</td>
<td>43%</td>
<td>42%</td>
<td>0.99</td>
</tr>
<tr>
<td>N2</td>
<td>KIAA0180</td>
<td>50%</td>
<td>43%</td>
<td>0.99</td>
</tr>
<tr>
<td>N7</td>
<td>HMG-CoA reductase</td>
<td>10%</td>
<td>25%</td>
<td>0.56</td>
</tr>
<tr>
<td>N8</td>
<td>EST AA592984 (LIBC)</td>
<td>20%</td>
<td>79%</td>
<td>0.0013a</td>
</tr>
<tr>
<td>N9</td>
<td>NOVEL</td>
<td>0%</td>
<td>0%</td>
<td>0.99</td>
</tr>
<tr>
<td>Transcripts expressed exclusively by the SUM149 IBC cell line</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>THC93623</td>
<td>57%</td>
<td>80%</td>
<td>0.433</td>
</tr>
<tr>
<td>T2</td>
<td>Deoxyhypusine synthase</td>
<td>84%</td>
<td>86%</td>
<td>0.99</td>
</tr>
<tr>
<td>T3</td>
<td>THC213005</td>
<td>57%</td>
<td>67%</td>
<td>0.719</td>
</tr>
<tr>
<td>T5</td>
<td>Overexpressed Br. Tu.a protein</td>
<td>83%</td>
<td>67%</td>
<td>0.569</td>
</tr>
<tr>
<td>T6</td>
<td>RhoC GTPase</td>
<td>90%</td>
<td>38%</td>
<td>0.0095a</td>
</tr>
<tr>
<td>T8</td>
<td>NOVEL</td>
<td>83%</td>
<td>86%</td>
<td>0.99</td>
</tr>
</tbody>
</table>

a Significant difference as determined by Fisher’s exact test.

b Br. Tu., breast tumor.

Bridization. Eleven antisense oligo probes were designed from the Northern blot probe sequences and shown to be specific by BLASTN database analysis. Not all transcripts identified by differential display were tested by in situ hybridization due to a lack of single-copy sequence to design unique and specific probes. The tumor samples were probed and scored blindly with respect to which group each sample belonged. Tumor specimens often contained normal mammary epithelial tissue and connective tissue, which acted as internal normal and negative controls for each probe, respectively. All experiments included an oligo-dT mRNA control and positive experimental control. With few exceptions, the normal tissue always stained positive when the samples were hybridized with probes for transcripts expressed in normal cells and rarely with tumor-derived transcript probes. Specimens and controls were tested randomly as described (18, 19, 20), and data assessed blindly by two separate investigators. The data were evaluated on the basis of frequency and intensity of expression in cells containing viable RNA as determined by the oligo-dT probe. A pathologist confirmed tissue type (i.e., normal versus tumor) for each specimen by comparison with a corresponding H&E-stained section. There was 100% concordance in the assignment of tumor and normal fractions between observers. The two groups of stage III samples were then segregated, and the percentages of tumors in each category that over- or underexpress each transcript are represented in Table 2. Significance in the difference of expression was determined using Fisher’s Exact test. A significant difference in the expression of the N8 transcript (a novel gene that we have termed LIBC; GenBank accession #AF143679; Fig. 3) was seen when comparing inflammatory and noninflammatory samples. LIBC was expressed in only 20% of inflammatory tumors, in comparison with 79% (P = 0.0013) of noninflammatory tumors. A difference in relative expression was also seen in transcript T6, RhoC GTPase, which was overexpressed in 90% of inflammatory tumors tested, in comparison with only 38% of noninflammatory tumors (P = 0.0095, Fig. 3). Although a low-level expression of RhoC was detected in a small percentage of normal tissues, staining intensity in the tumors was at least 5-fold greater. When comparing the concordance of having both these genes altered in advanced breast cancer, it was seen that loss of LIBC and overexpression of RhoC occurred in 91% of the inflammatory tumors assayed. In contrast, concordant alteration of both genes was not seen in any of the noninflammatory stage III breast cancers analyzed. The remaining transcripts were expressed in similar proportions by both inflammatory and noninflammatory tumors. Interestingly, N9, another novel gene, was not expressed in any tumor sample of either type, but was expressed in the normal mammary epithelial tissue of each of the samples, suggesting a putative tumor suppressor gene.

Identification of the LIBC Gene and Predicted Protein Structure. The full-length sequence of LIBC was determined by PCR-based screening of a random primed ovarian library and identified as a predicted gene within a PAC clone. The LIBC gene encodes a predicted 331 amino acid, 36.9-kDa protein. The predicted LIBC protein has 57% homology to human CTGF precursor. CTGF proteins are highly conserved throughout mammalian evolution; LIBC was seen to have 58%, 59%, and 56% homology to murine, porcine, and bovine CTGFs, respectively (Fig. 4). These proteins are members of the IGFBP family, and human CTGF has been described as a low-affinity IGFBP otherwise known as an IGFBP-rP (22). LIBC contains 36 cysteine residues and the IGF-binding domain, GCGCCKIC, starting at amino acid 48. Both protein kinase C and casein kinase 2 phosphorylation sites are present at the COOH-terminal domain. LIBC seems to be N-linked glycosylated and has several predicted myristilation sites throughout the protein that may associate LIBC with the plasma membrane and may aid in its interaction with IGF and insulin receptors.

DISCUSSION

These experiments provide new insights into the genetic alterations of IBC. In addition to the notably rapid growth rate of IBC, no typical precursor lesion of IBC has been described.
Fig. 3  *In situ* hybridization of serial sections of inflammatory and noninflammatory stage III archival breast cancer tumor samples with probes for LIBC and RhoC. LIBC expression was absent in inflammatory breast cancer, whereas RhoC was overexpressed. H&E staining of the specimens was used to identify tissue structure. An oligo-dT probe was used to demonstrate that the tissues had intact mRNA.
Because of this, it is our hypothesis that stage III IBC arises from a single or multiple genetic events occurring concurrently or in rapid succession. The rapid onset of the clinical presentation suggests that, in this disease, a limited number of genetic events may determine this aggressive phenotype.

Using this \emph{in vitro} model system, we compared a cell line derived from a primary IBC with that patient’s own immortalized lymphocytes and two immortalized replicating normal mammary epithelial cell lines. By this method, we were able to eliminate differences attributable to the immortalization process and expression differences possibly due to polymorphisms. In comparison, breast tissue procured from mammoplasty would provide a large amount of mRNA; however, the majority of cells from the normal breast epithelium would be in the resting state and would, thus, lead to isolation of transcripts that reflect the normal replicative process. Nonimmortalized HME cells grow in culture for two to four passages before becoming senescent. This greatly limits the number of experiments that can be performed on a particular set of normal cells. Additionally, the immortalized HME cells used in this study are well characterized with respect to their differentiation lineage, thus, ensuring that they are the normal counterpart to the SUM149 IBC cell line.

Seventeen genes, eight expressed exclusively by the normal cell lines and nine expressed by the tumor cell line, were identified. Two of the genes, \textit{LIBC} and the \textit{RhoC} GTPase were lost and overexpressed, respectively, in archival IBC samples when compared with noninflammatory stage III breast cancer. These two genes demonstrated a concordance of alteration in 91% of the inflammatory samples analyzed, and this pattern of alteration was not observed in any of the noninflammatory samples tested for both transcripts.

\textit{Rho} was first isolated and cloned from Aplysia and was found to be homologous to human (23). It is highly conserved throughout evolution, and, consequently, transfection of Aplysia Rho (which is 92% homologous to human RhoC) into NIH3T3 cells resulted in malignant transformation (24). The transforming ability of Rho family members in various other tissue culture systems has been documented (23, 24). Furthermore, overexpression of activated Rho proteins has been shown to be a requirement in ras-induced transformation of some fibroblast cell lines (25). Given that \textit{RhoC} participates in cytoskeletal reorganization, can modulate ras-dependent signal transduction, and control cellular proliferation and apoptosis (25, 26, 27, 28), it is a good candidate marker gene for the progression of an invasive and proliferative tumor, such as IBC. Recent studies have linked \textit{RhoC} overexpression with tumor progression of aggressive ductal adenocarcinoma of the pancreas and decreased survival (29). The exact mechanism of \textit{RhoC} overexpression is not yet known; however, neither gene amplification nor gain-of-function mutations have been found in our cell lines.

\textit{LIBC} is a strong candidate for a tumor suppressor gene in IBC because it seems to be a member of the low-affinity IGFBP family. Sequence homology and predicted protein structure of \textit{LIBC} suggest that it is a member of the low-affinity IGFs otherwise known as the IGFBP-rPs (30). At this time, should further characterization of \textit{LIBC} prove it to be an IGFBP-rP, it would be known as IGFBP-rP10. IGFBP-rPs seem to modulate the availability of IGFs to the IGF receptors and, therefore, regulate IGF-mediated proliferative and anabolic effects on the cells (31, 32). Additionally, recent studies have demonstrated that IGFBP-rPs may have IGF-independent effects on cellular growth because they specifically inhibit breast cancer cell proliferation (31, 33). Furthermore, Burger \textit{et al.} (34) have recently described IGFBP-rP1 (mac25/IGFBP-7), the down-regulation of which has been associated with progression of breast cancer. The same low-affinity IGFBP has been impli-
cated as a tumor suppressor protein in prostate cancer (35). This observation does not seem true for all of the IGFBPs. In the case of IGFBP-3, higher mRNA and protein levels in primary breast cancers are correlated with poor prognosis (36, 37). Taken together these data suggest a possible role for LIBC as a novel tumor suppressor belonging to the family of IGFBP-rPs.

Because no other statistically significant differences in expression between the inflammatory and noninflammatory phenotype were seen for the other genes in the panel, differences in expression of the remaining transcripts could represent genetic or epigenetic alterations common in advanced breast cancer.

The remainder of the genes described in this study, although not specific for IBC, may play a role in breast cancer development and progression. For those transcripts that have matches in the database to known genes, a clear role in cancer has yet to be defined. H-Nuc/cdc27 has been shown to be associated with the retinoblastoma tumor suppressor gene and is involved with cell cycle regulation (38, 39). Mutant H-Nuc/cdc27 has been described as a tumor-specific antigen recognized by CD4+ T cells (40). Deoxyhypusine synthase activates elF-5A by hypusinating a unique lysine residue on elF-5A (41). In turn, activated elF-5A is involved in protein synthesis (41). Chen and Chen (42) have demonstrated that v-HA-Ras-transformed cells have elevated hypusine formation on elF-5A (42). A growing body of evidence has demonstrated that genes such as those coding for ribosomal proteins (N3 and T9) modulate a variety of cellular functions, such as control of apoptotic pathways (43). Therefore, it is conceivable that alterations in one or more of these genes may contribute to breast tumor progression.

Of the genes that exhibit differential expression with respect to normal cells but are not specific for the inflammatory phenotype, HMG-CoA reductase merits specific attention. Alterations in expression of HMG-CoA reductase have been suggested to play a role in breast cancer progression (44, 45, 46). Interestingly, growth inhibition can be overexpressed in elF-5A (41). Chen and Chen (42) have demonstrated that v-HA-Ras-transformed cells have elevated hypusine formation on elF-5A (42). A growing body of evidence has demonstrated that genes such as those coding for ribosomal proteins (N3 and T9) modulate a variety of cellular functions, such as control of apoptotic pathways (43). Therefore, it is conceivable that alterations in one or more of these genes may contribute to breast tumor progression.

The function of these two genes as a tumor suppressor and oncogene, respectively, suggests new targets for therapeutic intervention. In addition, known genes such as HMG-CoA reductase, H-Nuc/cdc27, and deoxyhypusine synthase may be involved in breast cancer progression. Lastly, a novel putative tumor suppressor gene (N9) localized to chromosome 18q12 is frequently involved in locally advanced breast cancer. The experimental strategies followed to arrive at these markers may prove of general applicability to discern specific genetic factors in the pathogenesis of breast cancer subtypes for which appropriate cell lines are available. Up to now, this has been a major challenge in the molecular genetics of breast cancer, for which an orderly progression of lesions is difficult to discern.

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REFERENCES


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