Molecular Heterogeneity of Inflammatory Breast Cancer: A Hyperproliferative Phenotype

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Abstract

Purpose: Inflammatory breast cancer (IBC) is associated with very poor prognosis. The aims of this study are (*a*) to prospectively identify differential gene expression patterns associated with IBC and (*b*) to confirm these pathways using tissue arrays.

Experimental Design: For gene expression analysis, IBC ($n = 14$) was clinically defined as rapid-onset cancer associated with erythema and skin changes, whereas non-IBC patients ($n = 20$) had stage III breast cancers, and cDNA analysis was carried out using the Affymetrix (Santa Clara, CA) HG-U133A microarrays. Tissue arrays were constructed from paraffin-embedded material, and the molecular phenotype of 75 IBC was compared with results from >2,000 non-IBC.

Results: Gene expression analyses indicated that IBC has higher expression of genes associated with increased metabolic rate, lipid signaling, and cell turnover relative to non-IBC tumors. Consistent with the expression analysis, IBC had statistically higher Ki-67 (93% versus 11%; $P < 0.001$). BAX expression, reflecting increased apoptosis and cell turnover, was significantly uniformly higher in almost all IBC (98% versus 66%; $P < 0.05$), whereas the expression of Bcl-2 was not significantly different. IBC tumors were more likely to be steroid hormone receptor negative (estrogen receptor, 49% versus 30%; $P = 0.002$; progesterone receptor, 68% versus 42%; $P = 0.001$). The expression of tyrosine kinases was not significantly different. E-cadherin was found to be expressed in 87% of IBC, whereas the expression p53 was not significantly different.

Conclusion: This study is one of the largest molecular analyses of IBC. Both IBC and non-IBC are genetically heterogeneous with consistent differences in the molecular phenotype of IBC.

Breast cancer is complex and heterogeneous, with a range of overlapping clinical phenotypes that manifest a wide variation in prognosis and outcome. Inflammatory breast cancer (IBC) is a rare form of invasive breast cancer characterized clinically by rapid onset of breast erythema, edema, and ridging, with the time from first symptom/sign to diagnosis of ≤3 months (1). Until the advent of systemic therapy, this disease was almost universally fatal. Approximately 20% of patients with IBC have gross distant metastases at the time of diagnosis (2), in contrast to <5% of invasive ductal carcinomas of no special type. The mean 5-year survival rate in most studies of patients with IBC with local therapy alone is <5%, with median survival from 12 to 36 months (3). This is in contrast to no-special-type breast cancer where the 5-year survival exceeds 50% with local treatment alone. Thus, with local therapy alone, the probability of surviving IBC is only one-tenth that of other invasive breast cancers. Hence, IBC provides an ideal clinical window to study the molecular events that contribute to metastases and poor survival outcome. However, little is known about the molecular biology of this unusual and lethal variant of invasive breast cancer. There has been limited research done on the genetic alterations involved in its pathogenesis and progression largely due to its rarity. Additionally, the incidence of IBC seems to be increasing, lending support to the idea that environmental factors may contribute to this phenotype (4).

The biology of IBC has been reported in small patient numbers, with lower rates of estrogen receptor (ER) and progesterone receptor (PgR) expression, and faster growth kinetics than no-special-type cancers (5). Other key genes involved in carcinogenesis, such p53, pS2, RhoC, and HER-2, have shown varied levels of alteration in IBC (6). Better understanding of the events that drive the unusually aggressive biology of this special type of breast cancer may help in discovering relevant and important genetic pathways of metastases. To obtain further insight into the tumor genetic mechanisms driving the rapid clinical progression of IBC and the underlying metastasis-promoting processes, we first identified IBC-associated pathways by analyzing differential breast tumor gene expression with cDNA microarrays. We then confirmed some of the molecular pathways associated with

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IBC by immunohistochemical analysis in a large cohort of patients identified from the Baylor Tumor Bank. The aim of this study is to begin to establish the molecular phenotype of IBC that may explain the extraordinary metastatic potential associated with IBC.

Patients and Methods

Patients

There are two different patient populations in this study. Group 1: Tumor samples were obtained from a total of 14 IBC patients and 20 non-IBC reference patients with stage III breast cancer, identified from two prospective neoadjuvant chemotherapy clinical trials carried out at the Baylor Breast Center from 1999 to 2004. IBC patients were clinically defined by two experienced breast medical oncologists (J.C. and R.E.) as erythema, skin edema (peau d'orange), ridging, and rapid onset from first symptom/sign to diagnosis of ≤3 months. Non-IBC reference patients had stage III breast cancers without erythema. Total mRNA was extracted from the 14 IBC and 20 non-IBC reference patients and hybridized to high-density cDNA arrays. Group 2: A total of 75 IBC were retrospectively identified from the Baylor Tumor Bank. Eligible patients were initially diagnosed between 1970 and 1991 with primary breast cancers and were treated with surgery and adjuvant radiation therapy. More than 250 hospitals submitted tumor specimens to this Bank originally for steroid hormone receptor analysis. To obtain demographic information (age and menopausal status) and extent of disease, individual hospitals were contacted and retrospective chart reviews were requested for each patient. Patients were diagnosed with IBC based on clinical criteria as designated by the referring institution, and at least 100 mg of frozen tumor powder were required to be available for molecular analysis. Based on these criteria, we analyzed the molecular phenotype of 75 IBC and compared their molecular phenotype with previously analyzed results of >2,000 non-IBC obtained from the same tumor bank.

Methods

Gene expression array experiments. Core biopsies were taken from a total of 14 IBC and 20 non-IBC reference tumors before administration of neoadjuvant chemotherapy, snap frozen, deidentified, and fractionated into RNA, DNA, and protein. Total RNA from the frozen core biopsy specimens was isolated according to the protocols recommended by Affymetrix (Santa Clara, CA) for GeneChip experiments. Total RNA extraction was done using Trizol reagent (Invitrogen Corp., Carlsbad, CA). To remove RNA fragments less than 200 nucleotides, which comprised 15% to 20% of total RNA, each sample was passed over a Qiagen RNeasy column (Qiagen, Valencia, CA). A chimeric oligo(dT)-T_7 RNA polymerase promoter cDNA synthesis primer was then used to prepare double-stranded tumor cDNA. Reverse transcription was done on the cDNA to yield cRNA. Amplification and biotin labeling of antisense RNA was done using biotinylated ribonucleotides followed by cleanup of the reverse transcription. After chemical fragmentation, biotin-labeled cRNA was hydridized to Affymetrix HG-U133A microarrays. After automated washing and staining with streptavidin-phycoerythrin and biotinylated anti-streptavidin antibody (Vector Laboratories, Burlingame, CA), the arrays were scanned by Affymetrix GeneChip scanner (Agilent, Palo Alto, CA) and quantitated with Microarray suite (Affymetrix).

Statistical analysis. We used three software packages: dChip (http://www.dchip.org), BRB Array Tools (http://linus.nci.nih.gov/BRB-ArrayTools.html), and Array Analyzer (http://www.insightful.com). After scanning and low-level quantification using Microarray suite (http://www.affymetrix.com), we used dChip for normalization and estimation of expression values using the Li et al. PM-only model (7). The advantage of dChip is a model-based approach, which allows saving a model and applying it for individual new patient data. We used the PM-only model, because different studies found that it gives more precise expression estimations. We selected a subset of candidate genes by filtering on signal intensity to eliminate genes with uniformly low expression or genes whose expression did not vary significantly across the samples. BRB Array Tools and Array Analyzer were used for high-level analysis: identifying genes, Gene Ontology (GO) categories, and pathways differentially expressed between inflammatory and noninflammatory phenotypes.

We used t tests in BRB Array Tools (8) to find differentially expressed genes and two-way ANOVA in Array Analyzer (9) to account for the effect of ER and HER-2 status. Functional class scoring (10) was used to find GO categories and pathways that are significantly differentially expressed between inflammatory and noninflammatory phenotypes. This method is more powerful than common overrepresentation analysis of gene lists based on individually analyzed genes. It includes computing statistics that summarize Ps (for comparison of two classes) for all (N) genes in a GO category of pathways and comparison of these summary statistics with empirical distributions in random samples of N genes obtained by resampling. The two statistics used in pathway and GO comparisons were (a) LS statistics (defined as the mean negative natural algorithm of the Ps of the appropriate single genes univariate test) and (b) KS statistics (defined as the maximum difference between i/N and p_i, where p_i is the ith smallest P of the univariate test).

Immunohistochemical analysis. We compared differences in the molecular phenotype of the 75 IBC with previously analyzed results from >2,000 non-IBC reference tumors from the Baylor Tumor Bank. The assays for the IBC and non-IBC were done at different times. The pathways studied involved steroid hormone dependence (ER and PgR), apoptosis-related molecules (Bcl-2 and BAX), proliferation (Ki-67), growth factor receptor pathways [epidermal growth factor receptor (EGFR) and HER-2], p53, and cell adhesion molecules (E-cadherin). The standard method for immunohistochemical analysis was followed with slight modifications for tissue arrays. A small amount (~100 mg) of fresh frozen pulverized tumor materials was formalin-fixed and then paraffin-embedded. From this material, 5 × 3–mm cores of cylinders of tissue were arranged 12 to a slide, and semiquantitative measurement of protein expression of multiple biomarkers was done. These tissue microarrays enabled semiquantitation of the expression of multiple proteins in a particular pathway or pathways to be studied simultaneously in a rapid and cost-efficient manner and facilitated the molecular profiling of many known proteins involved in cancer-related pathways. Table 1 summarizes the characteristics of the primary antibodies used. The slides were incubated with the primary antibodies as listed in Table 1, and secondary antibodies were then applied. The secondary antibodies were then linked to peroxidase-conjugated

Table 1. Antibodies for immunohistochemistry and cutoff for evaluation using the Allred et al. scoring system (11), where the total score = intensity + proportion score

	Primary antibody	Evaluation cutoff points
ER	Abbott (ER-IC)	>2
PgR	Abbott (PR-ICA)	>2
HER-2	Zymed (tab 250)	>5
EGFR	Triton (31G7)	>0
p53	Novocastra (DO7)	>0
Bcl-2	DAKO (124)	>0
BAX	Zymed (2D2)	>0
E-cadherin	Zymed (HECD-1)	>0
Ki-67 high	Immunotech (Mib1)	>9*

*% Positive cells.

streptavidin. The chromogen signal was developed with 3,3′-diaminobenzidine, enhanced with osmium tetroxide, and contrasted to a 1% methyl green counterstain.

Immunostained slides were evaluated under a light microscope by the study pathologists (S.M. and D.C.A.) without any knowledge of the patients' data. The signal was scored using a system estimating both the proportion and the average intensity of positive tumor cells as described previously (11). The proportion of positive staining cells on the entire slide was scored as follows: 0, none; 1, proportion <1:100; 2, proportion 1:100-1:10; 3, proportion 1:10-1:3; 4, proportion 1:3-2:3; 5, proportion >2:3. The intensity of the positive signal was scored as follows: 0, negative; 1, weak staining; 2, intermediate staining; 3, strong staining. The overall score was expressed as a summation of the proportion and intensity scores. Tumors were regarded as expressing the particular molecular marker if the overall score was >2 for ER and PgR, >0 for p53, >0 for Bcl-2, >0 for BAX, >0 for EGFR, >0 for E-cadherin, and >5 for HER-2. For Ki-67, the percentage of positive staining cells was determined by direct counting, and staining in >9% of tumor cells was considered high proliferation (12).

Results

Gene expression array results. The characteristics of the 14 IBC patients and 20 non-IBC patients included in the gene expression array study are shown in Table 2. Of these, 13 IBC and 12 non-IBC samples were included in the final analysis after quality-control analysis. No statistically differences were found between IBC and non-IBC patients with respect to menopausal status, nodal status, grade, ER, PgR, or HER-2 status, although the statistical power to detect meaningful differences is relatively low due to small sample sizes. Considerable heterogeneity existed in both IBC and non-IBC, with weak signatures on individual gene-by-gene analyses and high false discovery rates. However, as patterns of gene expression rather than individual genes are more likely to discriminate between IBC and other cancers, we next did functional class scoring to discover GO categories and pathways that are significantly different between the two subtypes. For these analyses, statistical significance was defined as $P \leq 0.005$.

There were 16 pathways and 61 GO categories that significantly discriminated between IBC and non-IBC. The pathways involved in IBC were likely to have differential expression of fatty acid, glycerophospholipid, glycerosphingolipid, and inositol phosphate metabolism, bile acid and steroid biosynthesis, vesicle-associated cytoskeleton, extracellular matrix, glycolysis/glucogenesis, amino acid degradation and mTOR pathway, ribosome and mRNA processing, cell growth

Table 2. Characteristics of the 25 patients included in the gene expression analysis

	IBC (*n* = 13)	Non-IBS (*n* = 12)	*P*
Menopausal status			
Premenopausal	7	5	NS
Postmenopausal	6	7	
Node status			
n = 0	8	4	NS
n > 0	5	8	
Grade			
1-2	4	5	NS
3	4	6	
ND	5	2	
ER status			
Negative	11	7	NS
Positive	2	5	
PgR status			
Negative	10	8	NS
Positive	3	4	
HER-2 status			
Negative	4	7	NS
Positive	9	5	

Table 3. Discriminatory Cancer Genome Anatomy Project pathways between IBC and non-IBC, with permutation $P < 0.005$

	Pathway ID	Pathway description	No. genes	LS permutation *P*	KS permutation *P*
1	KEGG: hsa00071	Fatty acid metabolism	73	1e−05	0.00935
2	KEGG: hsa00280	Valine, leucine, and isoleucine degradation	45	1e−05	0.00448
3	KEGG: hsa00650	Butanoate metabolism	33	9e−05	0.01313
4	KEGG: hsa00062	Fatty acid biosynthesis (path 2)	16	0.00032	0.00126
5	KEGG: hsa00053	Ascorbate and aldarate metabolism	10	0.00035	0.02172
6	KEGG: hsa00640	Propanoate metabolism	39	0.00099	0.02776
7	KEGG: hsa00120	Bile acid biosynthesis	27	0.00122	0.03894
8	KEGG: hsa00310	Lysine degradation	40	0.00234	0.12269
9	KEGG: hsa03020	RNA polymerase	30	0.00349	0.08216
10	BioCarta: h_LairPathway	Cells and molecules involved in local acute inflammatory response	15	0.00366	0.03092
11	BioCarta: h_tcytotoxicPathway	T cytotoxic cell surface molecules	22	0.00374	0.02253
12	KEGG: hsa00330	Arginine and proline metabolism	52	0.00422	0.09767
13	BioCarta: h_ephA4Pathway	Eph kinases and ephrins support platelet aggregation	9	0.01216	0.00446
14	KEGG: hsa00920	Sulfur metabolism	11	0.01323	0.00348
15	BioCarta: h_PDZsPathway	Synaptic proteins at the synaptic junction	23	0.0545	0.00039
16	KEGG: hsa00910	Nitrogen metabolism	29	0.25434	0.004

Abbreviation: KEGG, Kyoto Encyclopedia of Genes and Genomes.

and differentiation, detoxification/cellular, stress and T-cell inflammatory response (Table 3). Consistent with the pathways, 61 significant GO categories discriminated between the two classes. These included genes involved primarily in metabolism and catabolism, cell turnover, amino acid metabolism, mRNA processing, protein transport, and immune response (Table 4). Although both sets of tumors overexpressed cell adhesion/signaling genes, IBC overexpressed the Alzheimer's disease–associated APP and γ-secretase constituents presenilin and anterior pharynx defective 1 homologue A, which also can proteolytically activate CDK5, Notch, Wnt, and HER4 pathway signaling. IBC also overexpressed extracellular matrix–associated CTSC, CTSF, CSPG2, and laminins relative to membrane cytoskeleton-associated integrins, EDIL3, SPTBN1, MAL, PROC, and KRT12 overexpressed in the non-IBC tumors. Overall, the results indicate higher metabolic rate, bioactive lipid signaling, and cell turnover of IBC relative to non-IBC tumors, with no statistically significant differences in steroid hormone receptor profiles.

We next investigated whether the 500 "intrinsic genes" first published by Sorlie et al. could discriminate different molecular subtypes of IBC and non-IBC (13). Unsupervised analysis revealed clusters in three major categories for both IBC and non-IBC: "ER" cluster, basal-like cluster, and HER-2 cluster (Fig. 1), reflecting that the heterogeneity and expression signatures inherent in non-IBC also exist with IBC.

The dChip "genome" routine, which statistically assesses the map distribution of differentially expressed genes in IBC versus non-IBC, detected several significant "blocks" of nonrandom gene mapping at breast tumor loss of heterozygosity–associated loci, suggesting chromosomal or DNA copy number alterations (Fig. 2). Most, such as the chromosome 1q, 5q, and 6p "blocks," are composed of genes from both tumor types, suggesting tumor genetic processes common to the IBC versus non-IBC tumors. However, two large "blocks" at breast cancer loss of heterozygosity–associated chromosome 14q24 and 19p13 loci are exclusively composed of genes overexpressed in IBC, suggesting that tumor genomic mechanisms contribute to the differential gene expression and phenotype of the these breast tumor types (Fig. 2).

Immunohistochemical results. The development of tissue array technology provides methodology for high-throughput concomitant analyses of multiple proteins on many archival tumor samples. Following the gene expression results, we compared by immunohistochemistry a total of 75 IBC with >2,000 non-IBC reference breast cancers from the tumor bank for the following to reflect pathways involved in cell turnover, catabolism, and metabolism as well as steroid hormone

Table 4. GO categories that discriminate IBC and non-IBC, with permutation $P < 0.005$

	GO category	GO description	No. genes	LS permutation *P*	KS permutation *P*
1	0003823	Antigen binding	34	1e−05	1e−05
2	0006525	Arginine metabolism	9	0.00012	0.001
3	0006787	Porphyrin catabolism	7	0.00019	1e−04
4	0030333	Antigen processing	35	0.00036	1e−05
5	0006527	Arginine catabolism	7	0.00036	0.00146
6	0004129	Cytochrome *c* oxidase activity	26	0.00038	0.00304
7	0015002	Heme-copper terminal oxidase activity	26	0.00038	0.00304
8	0016675	Oxidoreductase activity, acting on heme group of donors	26	0.00038	0.00304
9	0016676	Oxidoreductase activity, acting on heme group of donors, oxygen as acceptor	26	0.00038	0.00304
10	0000051	Urea cycle intermediate metabolism	10	6e−04	0.00296
11	0016813	Hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in linear amidines	7	0.00086	0.00168
12	0019882	Antigen presentation	44	0.00094	1e−05
13	0050660	FAD binding	9	0.00103	0.00602
14	0019883	Antigen presentation, endogenous antigen	17	0.00109	1e−05
15	0019885	Antigen processing, endogenous antigen via MHC class I	17	0.00109	1e−05
16	0000313	Organellar ribosome	25	0.00131	0.00315
17	0005761	Mitochondrial ribosome	25	0.00131	0.00315
18	0006778	Porphyrin metabolism	25	0.00154	0.01142
19	0044270	Nitrogen compound catabolism	9	0.00159	0.01364
20	0008147	Structural constituent of bone	6	0.00185	0.02226
21	0046483	Heterocycle metabolism	57	0.00211	0.03156
22	0050662	Coenzyme binding	23	0.00228	0.01673
23	0006081	Aldehyde metabolism	12	0.00299	0.18169
24	0004062	Aryl sulfotransferase activity	6	0.00335	0.00038
25	0045012	MHC class II receptor activity	23	0.00361	1e−05
26	0008194	UDP-glycosyltransferase activity	59	0.00393	0.03168
27	0019835	Cytolysis	5	0.00394	0.07823
28	0015020	Glucuronosyltransferase activity	11	0.00398	0.00913
29	0005750	Respiratory chain complex III (sensu Eukaryota)	5	0.00398	0.00451
30	0006743	Ubiquinone metabolism	5	0.00399	0.00398

(*Continued on the following page*)

receptors and known signaling pathways: ER, PgR, Ki-67, HER-2, EGFR, p53, Bcl-2, BAX, and E-cadherin. Table 5 summarizes the results obtained from the immunohistochemical experiments. Consistent with the expression analyses, IBC had statistically higher proliferation as measured by Ki-67 (93% of IBC samples had high proliferation versus 11% of non-IBC samples; $P < 0.001$). BAX expression, reflecting increased apoptosis and cell turnover, was significantly more frequent in almost all IBC (98% versus 66%; $P < 0.05$), whereas the expression of Bcl-2 was not significantly different (63% versus 68%). IBC tumors were more likely to be ER negative (49% versus 30%; $P = 0.002$) and PgR negative (68% versus 42%; $P = 0.001$). The expression of signaling tyrosine kinases was not significantly different (EGFR positive, 23% versus 19%; HER-2 positive, 26% versus 17%) between IBC and non-IBC samples. E-cadherin was found to be expressed in 87% of IBC but was not evaluated in non-IBC. In contrast, p53 was expressed significantly more often in non-IBC samples (52%) compared with IBC samples (32%; $P = 0.002$; Table 5).

Discussion

This study is one of the largest comprehensive molecular analyses of IBC, using both gene expression arrays and tissue arrays. The results showed that both IBC and non-IBC tumors are genetically heterogeneous, with molecular phenotypes as previously described inherent in both tumor types (13), and some consistent differences exist in IBC compared with non-IBC.

Gene expression analyses indicated that genomic mechanisms contribute to the phenotype of IBC, with nonrandom regions of differential genes associated with IBC. IBC has also higher expression of genes associated with higher metabolic rate, bioactive lipid signaling, and cell turnover of IBC relative to non-IBC tumors. Consistent with these results, tissue array analyses confirmed that IBC tumors were rapidly proliferating, with nearly all tumors showing elevated Ki-67, and with increased apoptotic markers like BAX. Ki-67 is a nuclear endogen expressed only in proliferating cells (late G_1, S, M, and G_2) and is associated with poorer prognosis (14). Nearly all IBC had a high proliferation fraction, 93% versus 11% of non-IBC. This is also consistent with the clinical observation of rapid onset in IBC together with poorer prognosis and outcome compared with other locally advanced breast cancers. Consistent with increased cell turnover, BAX was significantly increased in IBC. The proapoptotic activity of BAX is neutralized when BAX is bound to Bcl-2 or other members of the Bcl-2 family (e.g., Bcl-X_L; refs. 15, 16). From these results,

Table 4. GO categories that discriminate IBC and non-IBC, with permutation $P < 0.005$ (Cont'd)

	GO category	GO description	No. genes	LS permutation *P*	KS permutation *P*
31	0006744	Ubiquinone biosynthesis	5	0.00399	0.00398
32	0042375	Quinone cofactor metabolism	5	0.00399	0.00398
33	0045426	Quinone cofactor biosynthesis	5	0.00399	0.00398
34	0016782	Transferase activity, transferring sulfur-containing groups	32	0.00401	0.00066
35	0005583	Fibrillar collagen	18	0.00422	0.00038
36	0051017	Actin filament bundle formation	5	0.00429	0.14763
37	0004029	Aldehyde dehydrogenase (NAD) activity	12	0.00429	0.17928
38	0006354	RNA elongation	9	0.0043	0.03916
39	0008146	Sulfotransferase activity	28	0.00452	0.00015
40	0007585	Respiratory gaseous exchange	13	0.00474	0.0064
41	0000050	Urea cycle	6	0.00481	0.02556
42	0008021	Synaptic vesicle	20	0.00554	0.00043
43	0008210	Estrogen metabolism	5	0.00574	0.00065
44	0004075	Biotin carboxylase activity	6	0.00824	0.00037
45	0007269	Neurotransmitter secretion	5	0.0111	0.00155
46	0016421	CoA carboxylase activity	6	0.01226	0.00017
47	0005540	Hyaluronic acid binding	17	0.01236	0.00244
48	0016885	Ligase activity, forming carbon-carbon bonds	7	0.01367	0.00015
49	0016706	Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors	7	0.01438	0.00125
50	0006584	Catecholamine metabolism	14	0.01789	0.00275
51	0018958	Phenol metabolism	14	0.01789	0.00275
52	0005581	Collagen	34	0.01902	0.00085
53	0000096	Sulfur amino acid metabolism	24	0.01908	0.00125
54	0019884	Antigen presentation, exogenous antigen	17	0.03115	0.00044
55	0004385	Guanylate kinase activity	13	0.04164	0.00396
56	0016282	Eukaryotic 43S preinitiation complex	37	0.04445	0.00087
57	0019886	Antigen processing, exogenous antigen via MHC class II	18	0.04481	0.00192
58	0006941	Striated muscle contraction	18	0.05668	0.00382
59	0006817	Phosphate transport	48	0.06297	1e−05
60	0016805	Dipeptidase activity	6	0.07543	0.00398
61	0015698	Inorganic anion transport	89	0.12696	0.00122

Table 5. Tissue arrays comparing 75 IBC with >2,000 non-IBC: immunohistochemistry results

	Inflammatory (n = 75), %	Non-IBC (n = 2,093), %	P
ER−	49	30	0.002
PgR−	68	43	0.001
HER-2+	17	26	0.09
EGFR+	23	19	0.4
p53 expression	32	52	0.002
Bcl-2 expression	63	68	0.5
BAX expression	98	66*	<0.05
E-cadherin expression	87	—†	—†
High Ki-67	93	11	0.001

*n = 200.
†E-cadherin expression was not done on non-IBC.

this apoptotic pathway may be important therapeutic target in improving outcome in patients with IBC.

The p53 tumor suppressor gene has several functions affecting cell turnover, including activating the cell cycle by activating p23 and also promoting apoptosis (17). Mutation and loss of p53 function are associated with high proliferation rates and poor clinical outcome. Based on the high proliferation rates in IBC, we might have predicted p53 accumulation in IBC. Contrary to this expectation, we found p53 accumulation in approximately one-third of IBC cancers and half of non-IBC tumors (32% versus 52%). These results may indicate that the high cell turnover in IBC could be driven by mechanisms other than altered p53 or that p53 may be excluded from the nucleus and therefore may be nonfunctional (18).

Gene expression analyses did not show higher expression of inflammatory components in IBC compared with non-IBC group, consistent with the thinking that the erythema in IBC results from blockage of the lymphatic channels with tumor emboli rather than direct infiltration of the skin by inflammatory cells (19). Our results are consistent with other studies showing expression of E-cadherin in ~90% of IBC (20). E-cadherin is thought to be a tumor suppressor gene, and its absence or low expression has been associated with high histologic grade, increased invasiveness, and high metastatic potential (21). Its conserved expression in IBC, although unexpected, has been described previously (20, 22, 23). A possible explanation for this paradoxical conservation of E-cadherin might be that the loss of E-cadherin is only transient and associated with the transit of cancer cells into the lymphovascular system. Once in the circulation, the cancer cells may reinstate the expression of E-cadherin, thereby facilitating intracellular adhesion and the formation of tumor emboli (19). Our observation that E-cadherin is frequently expressed in IBC might support the possibility that its expression may be important in the formation of tumor emboli.

Surprisingly, RhoC was not overexpressed in our group of IBC tumors, as published previously (6), but are, however, consistent with results by Bieche et al. (24). RhoC GTPase is a member of the Ras superfamily of small GTP-binding proteins. It has been shown to enhance invasiveness and motility, and this apparent discrepancy may arise from the genetic heterogeneity of IBC.

The importance of steroid hormone receptors has been well established in breast cancer. Estrogen, acting through the ER, promotes breast cancer growth and development. ER is expressed in 60% to 70% of invasive breast cancers and is associated with low proliferation rate, better response to endocrine therapy (25), and a lower risk of relapse (26, 27). Interestingly, immunohistochemical analysis showed that although IBC was slightly more likely to be ER negative and PgR negative, a third to a half still expressed these receptors. The growth factor receptors EGFR and HER-2 are often associated with poorer prognosis and increased tumor proliferation (28, 29), but from our results it is unlikely that the EFGR/HER-2 family is primarily responsible for the increased proliferation of IBC.

One of the limitations of this project is in the selection of IBC from the Baylor Tumor Bank. It is uncertain how many of the 75 IBC cases from the tumor bank actually fulfilled the clinical goal standard definition of IBC, because these cases represent assignment of IBC by the referring "community" physicians. Another limitation is that the non-IBC samples may not be true controls, because a portion of these may have included lower-grade and early-stage cancers.

We compared our results with two previously published differentially expressed genes for IBC (22). Of the 90 differentially expressed genes in the Bertucci et al. article (22), only kinase anchor protein 1 (PRKA) was significantly differentially expressed in our data set. Of the 50 differentially expressed genes from the Van Laere et al. article (30), only 5 genes overlapped (hemochromatosis, platelet-derived growth factor-α polypeptide, selectin P ligand, nucleotide binding protein 1, and cell division cycle 25B). Interestingly, little overlap in gene expression was observed between these two earlier studies, further supporting our hypothesis that

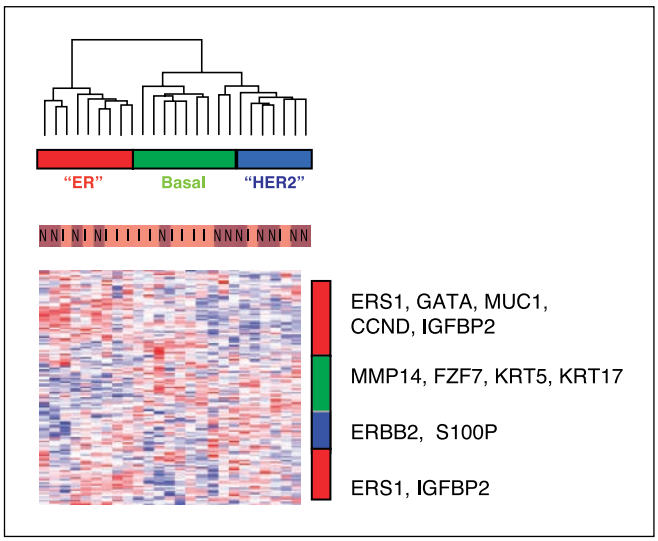

Fig. 1. Hierarchical clustering of expression data from Sorlie et al. *Rows,* genes; *columns,* samples. Expression level of each gene in a single sample is relative to its median level across all IBC (*I*) and non-IBC (*N*) samples. *Red* and *blue,* expression levels above and below the median, respectively. The dendrogram of samples represents overall similarities in gene expression profiles. Under the dendrogram, the horizontal colored boxes delimit the different subgroups: ER-like (*red box*), basal (*green box*), and HER-2-overexpressing (*blue box*). Branches of the core samples for each of the centroids are similarly color coded. *Colored bars, right,* locations of three gene clusters of interest: ER (*red bar*), basal (*green bar*), and HER-2 cluster (*blue bar*). Some genes included in these clusters are referenced by their HUGO abbreviation as used in Locus Link.

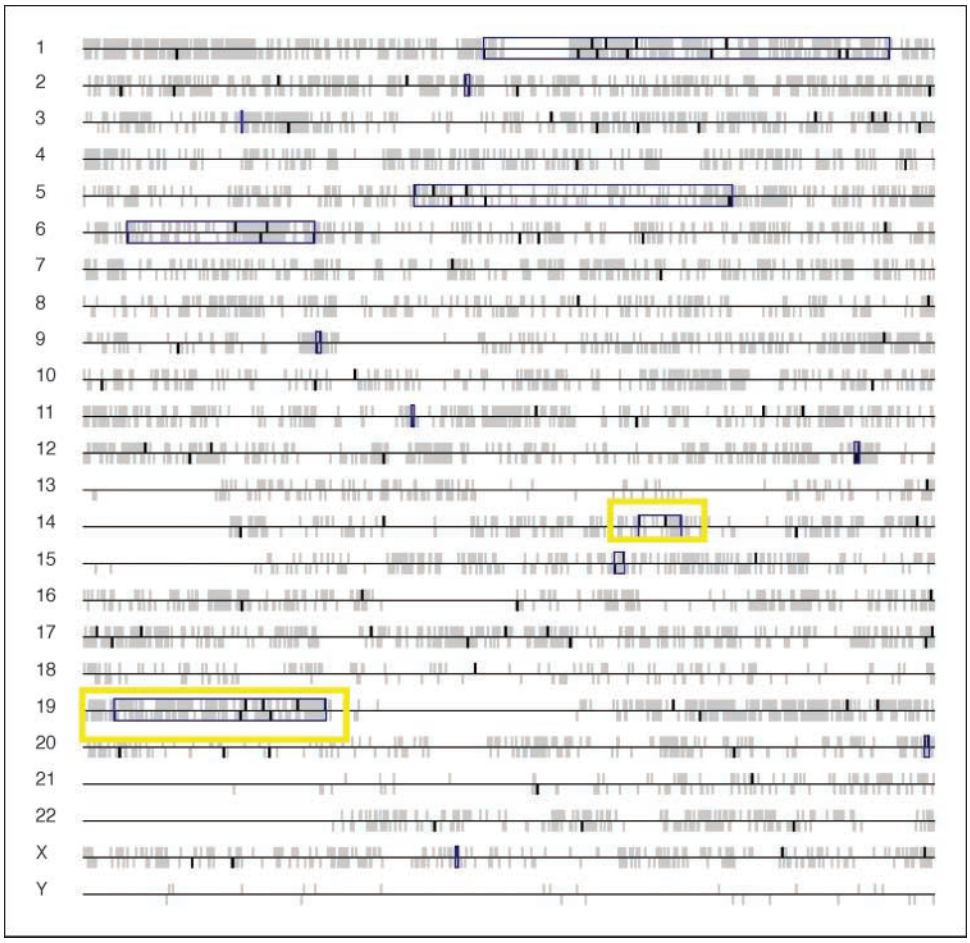

Fig. 2. dChip "genome" map showing the distribution of significant differentially expressed genes in IBC versus non-IBC. Significant "blocks" of nonrandom gene mapping at breast tumor loss of heterozygosity – associated loci were found at chromosome 14q24 and 19p13 loci (*yellow boxes*) in IBC, suggesting chromosomal or DNA copy number alterations.

IBC express a hyperproliferative profile with genetic heterogeneity.

Breast cancer is a clinically diverse disease, and this diversity is driven by multiple genetic alterations and molecular events. IBC, with its poor prognosis and distinct clinical manifestation, is a hallmark of this genetic diversity. Using small amounts of material, we have used tissue arrays and gene expression analysis to examine the tumor-genetic mechanisms that might contribute to the extraordinary malignancy of IBC. Mirroring their distinct clinical behavior, IBC have a profile that is hyperproliferative and seem to be driven by several diverse pathways. The Bcl-2/BAX apoptotic pathway may be an important target. From our results, pathways especially those involved in fatty acid and lipid metabolism seem to be important, and interventions, including life-style modifications, may improve outcome. We are continuing to refine this profile further and to investigate its implications for biology and treatment.

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