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Different Biological Processes are Associated with the Different Molecular Subtypes of Inflammatory Breast Cancer

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Abstract

Purpose—The goal of this study was to determine whether gene expression differences exist between inflammatory breast cancers (IBC) and stage-matched non-IBC patients stratified by hormone receptor and HER2 status.

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Experimental Design—We used Affymetrix GeneChips to analyze 82 tumor samples (25 T4d, 25 patients and 57, T4a-c patients) of newly diagnosed breast cancers. Genes that were differentially expressed between the IBC and non-IBC specimens were identified using the t-test, and differential expression of gene sets was assessed using gene set analysis. Three distinct clinical subtypes of IBC and non-IBC were compared: ER-positive/HER2-normal, HER2-amplified, and ER-negative/HER2-normal.

Results—When we compared expression data from all IBC with all non-IBC, we found no significant differences after adjusting for multiple testing. When IBC and non-IBC tumors were compared by clinical subtype, however, significant differences emerged. Complement and immune system-related pathways were overexpressed in ER-positive/HER2-normal IBC. Protein translation and mTOR signaling were overexpressed in HER2-amplified IBC. Apoptosis-, neural-, and lipid metabolism-related pathways were overexpressed in ER-negative/HER2-normal IBC compared with non-IBC of the same receptor phenotype. In this stage-matched case-control study, the survival curves of patients with IBC and non-IBC were similar for all three clinical subtypes.

Conclusions—IBC tumors can be divided into molecular and clinical subtypes similar to those of non-IBC. Clinical subtypes of IBC show molecular differences compared with similar subtypes of non-IBC.

Keywords

Inflammatory breast cancer; receptor subtypes; cDNA microarray; Gene set analysis

Introduction

Inflammatory breast cancer (IBC) is a clinical diagnosis designated as T4d disease in the TNM classification of the American Joint Committee on Cancer [1]. IBC is distinct from neglected, locally advanced breast cancer with a clinical course characterized by a rapid course of progression featuring local growth and early metastasis. IBC is a rare breast cancer that accounts for 1 to 5% of all breast cancers in the United States [2,3]. Several authors have analyzed gene expression profiles of IBC to evaluate peculiar features of this disease [4–7]. Van Laere et al. reported that the insulin-like growth factor-signaling pathway is implicated in the biology of IBC [7]. Nguyen et al. reported that IBC has higher expression of genes associated with increased metabolic rate, lipid signaling, and cell turnover [6]. All previous studies compared IBC with non-IBC simply on the bases of clinical diagnosis and without considering molecular subtypes of breast disease. However, breast cancer is no longer considered to be a single disease with variable receptor expression and histology but rather as a collection of several distinct diseases distinguished by unique clinical, biological and molecular features. These molecular and clinical subtypes can be discerned in both IBC and non-IBC.

We hypothesized that there are biological differences between IBC and non-IBC tumors according to each clinical subtype, including ER-positive/HER2-normal, HER2-amplified with any ER status, and ER–/HER2-normal cancers. We tested this hypothesis at the individual gene level and at the level of gene sets that represent biological functions by

comparing IBC and non-IBC within each clinical subtype. We also studied survival for patients with IBC and non-IBC tumors matched by clinical subtype.

Materials and Methods

Patient cohorts and gene expression data

Tumor biopsies were collected prospectively from patients with locally advanced disease who were candidates for neoadjuvant therapy. Fine needle aspiration (FNA) were obtained before administration of any systemic therapy as part of an ongoing pharmacogenomic research program at The University of Texas MD Anderson Cancer Center. All patients gave written informed consent for voluntary participation. The study was approved by the Institutional Review Board. Samples were placed in RNAlater (Ambion, Austin, TX) storage reagent and stored at -80°C until gene expression analysis. This study followed a case-control design. Patients with a clinical diagnosis of IBC were selected after reviewing all medical records associated with the 440 FNA samples with complete gene expression data. Data were collected between 2000 and 2007. IBC was defined as the presence of erythema and edema (peau d'orange) involving at least one third of the skin of the breast and rapid clinical presentation and biopsy-proven invasive breast carcinoma. The presence of tumor emboli in the dermal lymphatics of the involved skin in the pathology report was not required for inclusion as IBC. Controls were selected to match the T stage, with all T4a-c tumors in the data set included as controls. We identified 25 IBCs and 57 non-IBC controls for this analysis. Clinicopathologic characteristics of the patient cohorts are presented in Table 1.

Gene expression profiling was performed using Affymetrix U133A gene chips as previously described [8]. Gene expression data were normalized with the MAS5 algorithm, mean centered to 600 and log 2 transformed before further analysis. Probe sets with the lowest 15% mean expression value were removed from all higher level analyses to reduce noise from low expressed probe sets. This left 18,940 probe sets for analysis. Cases with normalized ESR1 mRNA expression (probe set 205225_at) >10.18 were considered ER positive, and cases with HER-2 mRNA expression (probe set 216836_s_at) >12.54 were considered HER2 amplified [9]. Molecular class was assigned based on gene expression profiles using the PAM 50 centroid-based classifier [10]. All statistical analysis was performed with BRB Array Tools version 3.9.0 Alfa software (<http://linus.nci.nih.gov/BRB-ArrayTools.html>) and R software version 2.7.2 (<http://www.r-project.org>). Complete gene expression data were deposited in the Gene Expression Omnibus (GEO) data base under GSE22597.

Identification of differentially expressed genes and gene sets

We compared gene expression data between IBC and non-IBC tumors without any further stratification by clinical or molecular class using the t-test. We also performed similar class comparisons separately for each of the three clinical subtypes (ER+/HER2-, ER-/HER2-, HER2+). To adjust for the multiple comparisons, we calculated False Discovery Rates (FDR) and also assessed Global Significance using BRB Array tools. The FDR was calculated with the Significance Analysis of Microarrays (SAM) tool as the median number

of false-positive genes from permutation testing divided by the number of nominally significant genes defined from the unperturbed data [11]. The Global P value is the probability of getting at least the same number of genes significant on a parametric test (e.g., t-test) at the specified P level by chance if there are no real differences between IBC and non-IBC. Significantly differentially expressed genes were also mapped to functional pathways using the Ingenuity Pathway Analysis software (IPA, <http://www.ingenuity.com/>).

We also examined differential expression of a priori defined gene sets using Gene Set Analysis (GSA). The goal of GSA is to determine whether members of a set of genes that correspond to a particular biological pathway tend to occur toward the top or the bottom of a rank-ordered gene list (rank ordered by differential expression between IBC versus non-IBC) [12]. In this analysis we included three gene sets that were previously reported to be discriminatory between IBC and non-IBC [13,4,14] or associated with poor prognosis in breast cancer, including a Wnt pathway gene set [15] and CD44+ related stem cell signature [16] (Supplementary Table 1). We also tested 2,113 different, functionally annotated gene sets from Gene Ontology (GO, <http://www.geneontology.org>) that collectively represent most known biological and metabolic pathways in human cells. Gene sets with a minimum number of 10 genes and maximum of 100 genes were selected for inclusion in this analysis. We used the Efron and Tibshirani gene set analysis method that employs “maxmean” statistics and is implemented by the BRB Array Tools [17,18]. Significance was estimated with the permutation test ($n = 1000$). The null hypothesis was that the average degree of differential expression of members of a given gene set between the IBC and non-IBC cohorts is the same as expected from a random set of genes of similar size.

Survival analysis

Survival was estimated by the Kaplan-Meier product limit method and compared between groups with the log-rank statistic. Relapse-free survival was defined as the time from the diagnosis to local, regional, or distant relapse. Fourteen patients with stage IV disease (three IBC and 11 non-IBC) and five additional patients who did not undergo surgery (one IBC and four non-IBC, stage III disease) were excluded from the relapse-free survival analysis. Overall survival was defined as the time from the diagnosis to death, and all patients were included in this analysis.

Results

Differentially expressed genes between IBC and non-IBC

Clinical characteristics of the 82 patients included in this study are presented in Table 1. The proportions of patients with lymphatic invasion (76% vs 32%), ER-negative disease (76% versus 46%) and HER2-amplified molecular class (40% vs 26%) were greater in the IBC cohort than in the T stage-matched non-IBC group. We first compared IBC with non-IBC regardless of their clinical subtypes. At any parametric P -value threshold, the Global significance test indicated that the findings were not different from what could be expected by chance alone (Table 2). Next, we performed the same analysis by clinical subtypes. Statistically significant differences using the Global test were observed in HER2-amplified cancers (Table 2 and Supplementary Table 2). Five hundred eight probe sets were

differentially expressed between HER2-amplified IBC and non-IBC at parametric $P = 0.01$ (Global $P = 0.04$): 171 probe sets were overexpressed and 337 were underexpressed in IBC. However, the FDR rates associated with individual parametric P -values ranged from 1.5% to 37%, indicating a substantial potential for false discovery among these genes. We mapped the 171 probe sets overexpressed in IBC to biological pathways, using Ingenuity Pathway Analysis. The top three pathways overexpressed in HER2-amplified IBC relative to non-IBC were eIF2 signaling, regulation of eIF4 and p70S6K signaling, and mammalian target of rapamycin (mTOR) signaling (Fig. 1A). Silvera et al. have reported that eIF4G1 reprograms the protein synthetic machinery for increased translation of mRNAs with internal ribosome entry sites that promote IBC tumor cell survival and formation of tumor emboli [19].

Gene Set Analysis (GSA) results

Individual gene (i.e., probe set) level analysis suggested relatively modest gene expression differences between IBC and non-IBC. Next, we investigated whether we could detect coordinated but relatively small scale differences in the expression of gene sets that belong to functional pathways. Such consistent but small-scale differences at the individual gene level may not be readily identified by t-statistics but gene set enrichment analysis may be able to identify them. We tested five gene sets that were previously reported to be implicated in breast cancer prognosis or in the biology of IBC [15,13,4,16,14]. None of these previously published gene sets showed significant differential expression when all IBC cases were compared with all non-IBC (Table 2). When comparisons were made within clinical subtypes, the IBC metagene by Bieche et al [4] was significantly higher in ER-positive/HER2-normal IBC than in non-IBC of the same phenotype. The gene set by Bertucci et al. [14] was significantly higher in HER2-amplified IBC and the CD44 stem cell signature had borderline significance. The Wnt gene set [15] was significantly overexpressed in ER-negative/HER2-normal IBC (Table 2). The Wnt gene set was identified in a signature that discriminates lung metastases from primary tumor in SUM1315 orthotopic xenografts. Interestingly, SUM1315 breast cancer cells were initially isolated from a breast cancer skin metastasis [20] and were reported to correlation with basal breast cancer molecular subtypes [15]. To examine possible differences between IBC and non-IBC over a broad range of biological processes, we also tested 2113 functionally annotated gene sets assembled from Gene Ontology. Many gene sets showed significant differential expression by IBC status within the three clinical subtypes (Supplementary Table 3 and Supplementary Table 4 1–4). At a P value of 0.01 (Efron-Tibshirani test), 28, 69, and 41 gene sets were differently expressed in ER-positive/HER2-normal, HER2-positive, and ER-negative/HER2-normal cancers, respectively. The gene sets that were significantly overexpressed in IBC in the three clinical subtypes are listed in Table 3. We mapped all the members of these overexpressed gene sets (17, 33, and 18 gene sets by each subtype) to biological pathways by IPA; the results are shown in Fig. 1B,C and D. In HER2-amplified IBC, the most significant pathways identified through GO were the same as those identified by the gene-by-gene analysis (Fig. 1A,B). In ER-positive/HER2-normal IBC, the most significant pathways overexpressed in this subtype were the complement system, oncostatin signaling, and various immune cell-related pathways (Fig. 1C). In the ER-negative/HER2-normal IBC specimens, Myc-mediated apoptosis signaling, axonal guidance signaling, and Neuregulin

signaling as well as some lipid metabolism-related pathways were overexpressed. These results suggest that subtle biological differences may exist between breast cancer subtypes that show the inflammatory phenotype compared with tumors of the same subtype without inflammatory features. Interestingly, these differences seemed to vary by subtype.

Subtype-specific survival of IBC and non-IBC

We examined whether the survival of patients with the three differs between IBC and non-IBC, with or without stratifying by clinical subtype. The median follow-ups were 53 months (range: 18.5–89.8) and 57.2 months (range: 9.6–112.5) for the IBC and non-IBC cohorts, respectively. In this case-control study, the overall survival results did not significantly differ for IBC and non-IBC, overall or within each clinical subset (Fig. 2). Among the IBC cases under consideration, 0 out of 5 patients with ER-positive/HER2-normal tumors, 6 (50%) of 12 with HER2-positive, and 5 (62.5%) of 8 with ER-negative/HER2-normal had died. Among the non-IBC cases, 5 (22.7%) out of 22 patients with ER-positive/HER2-normal tumors, 6 (35.3%) of 17 with HER2-positive, and 9 (50%) of 18 with ER-negative/HER2-normal had died. Relapse-free survival was worse in ER-negative/HER2-normal IBC patients than for the non-IBC of the same phenotype (Supplementary Fig. 1). There was also no significant difference in overall or relapse-free survival by clinical subtype between the IBC and non-IBC cohorts (Fig. 3). A trend was observed in both IBC and non-IBC for better survival in ER-positive/HER-normal cases, but the small sample limited the power of survival analysis by subtype. Also, survival analysis might have been affected by an imbalance for targeted therapy (trastuzumab: HER2 antibody). Six (50%) of 12 patients with HER2-amplified IBC and 11 (65%) of 17 patients with HER2-amplified non-IBC had never received either neoadjuvant or adjuvant therapy.

Discussion

This is the first study to systematically examine gene expression differences between IBC and non-IBC patients according to clinically relevant molecular subtypes defined based on ER and HER2 expression. Moreover, we also carefully matched the IBC and non-IBC cohorts for T stage. All patients in this analysis had clinical T4 and represented locally advanced breast cancer with and without inflammatory features. In a global comparison of IBC versus non-IBC, we detected no significant gene expression differences at the individual gene level between these two cohorts of cases after adjusting for multiple comparisons. This is in contrast to several published reports that found significant gene expression differences between IBC and non-IBC [4–7]. This could probably be related to differences in the clinical characteristics of the comparison cohort. A closer analysis of the published literature reveals that many of the previous reports have not included stage II cancers in the comparison cohort [4,5]. Some of the previous reports have not adjusted for multiple comparisons or used reverse transcription polymerase chain reaction (RT-PCR) to quantify gene expression differences, and RT-PCR has a different sensitivity and dynamic range of mRNA detection compared with gene expression arrays [4].

Importantly, all of these previous reports that suggested gene expression differences between IBC and non-IBC reported small-scale differences. One report assessed 538 genes

and identified 22 that were differentially expressed [4]. Another report evaluated over 8,000 genes and found 105 that were differentially expressed [5]. A third report, similar to our analysis, found no significant differences at the individual gene level but observed some differences at the gene set level. [6] A fourth group of investigators tested close to 6,000 genes and found 50 that could discriminate between IBC and non-IBC [7]. When we tested these previously identified gene sets, we could only partially confirm the results from these publications in the current data set. Several of the gene sets were significantly overexpressed in IBC but only in particular clinical subtypes and not during a global comparison of all IBC with all non-IBC cohorts (as previously reported). This discrepancy reflects the unstable nature of these observations due to small and variable sample sizes of all current studies and the potential confounding effect of a variable clinical subtype distribution from study to study. The numbers of IBC cases included in the comparisons in published studies ranged from 14 to 37, and all previous analyses compared IBC with non-IBC without adjusting for molecular class or clinical receptor phenotype. Subtle imbalances in receptor phenotype between the IBC and non-IBC cohorts could lead to the identification of significantly differentially expressed genes that reflect the large-scale gene expression differences between molecular classes that were unevenly distributed across the two comparison cohorts, rather than the IBC-specific gene expression differences.

In the current study, we compared IBC and non-IBC after stratifying both groups into three clinically relevant subtypes of breast cancers. We found that individual gene level analysis showed significant differences only within the HER2-amplified group. Indeed, the natural history of HER2-amplified breast cancer has been dramatically changed by treatment with trastuzumab, an anti-HER2 antibody [21,22]. This observation has also been noted in IBC [23–27]. Several studies have reported the efficacy of trastuzumab in the treatment of HER2-amplified IBC [23–26]. Kaufman et al. reported that treatment with an EGFR/HER2 tyrosine kinase inhibitor, lapatinib, provided clinical efficacy in HER2-amplified IBC during phase II trials [27]. For treatment of the other subtypes of IBC, no similar targeted drugs are available. We speculate the unique activity could stem from the different HER2 pathway involvement between IBC and non-IBC. In the other clinical subtypes, individual gene level analysis showed no significant differences.

When gene sets were tested for differential expression, different gene sets distinguished IBC from non-IBC in each clinical subtype (Fig. 1). This suggests the possibility that different biological pathways are involved with the pathogenesis of the inflammatory phenotype in different molecular subsets of breast cancers. We found that complement and immune systems were highly associated with the ER-positive/HER2-normal IBC phenotype. Activation and regulation of complement pathways have been reported to play important roles in inflammatory mediators [28]. In addition, we found that protein translation and mTOR signaling were overexpressed in HER2-amplified tumors. mTOR, which functions downstream of the PI3K/Akt signaling pathway, is also being explored in therapies for HER2-amplified breast cancer [29]. mTOR inhibitors being tested in clinical trials for patients with breast cancer and other solid tumors include CCI-779 [30], RAD001 [31], and AP23573 [32]. We speculate that the importance of the mTOR pathway could be tested as potential target for HER2-amplified IBC by using one of the mTOR inhibitors. On the other hand, GSA showed that apoptosis, neural, and lipid metabolism pathways were

overexpressed in ER-negative/HER2-normal IBC. There is biological plausibility to implicate these processes in IBC features. It is known that the neurotransmitter pathway and lipid metabolism play important roles in the regulation of cell migration and apoptosis [33–35]. These observations suggest the possibility of potential novel strategies to modulate ER-negative/HER2-normal IBC. However, the lack of overlap between the discriminating gene sets is also partly due to false-negative findings owing to the small sample sizes for each cohort, leading to limited power for detecting differentially expressed gene sets.

Our data suggest that future studies involving IBC should consider the various clinical subtypes separately. These subtypes may have important clinical and molecular differences that justify their separate analysis. The different clinical prognoses of patients with the different clinical subtypes of breast cancer are well recognized, and similar differences in outcome may also exist among patients with IBC. This current study did not find any difference in overall survival related to different clinical subtypes of IBC, but we observed a worse relapse-free survival for patients with ER-negative/HER2-normal IBC compared with non-IBC of the same phenotype. However, this study was limited in its power to detect any but the most dramatic differences in survival because of the small sample size, which is due to the rarity of this disease entity (particularly when IBC is further subdivided by clinical phenotype). It may be necessary to create national and international cooperative networks to ensure sufficient sample sizes and homogeneous and mutually compatible analysis platforms for future studies of IBC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Ingenuity Pathway Analysis (IPA) software results showing the top 10 canonical pathways by significant genes overexpressed in IBC. The bars indicate $-\log(P\text{-value})$, calculated by Fisher's exact test. The line indicates the ratio for the percentage of genes in a pathway that were found in our uploaded list. Threshold indicates the line at $P = 0.05$.

Fig. 2. Kaplan-Meier curves showing overall survival by breast cancer phenotype for each receptor status.

Fig. 3. Kaplan-Meier curves showing overall survival by receptor subtype for each breast cancer phenotype.

Table 1

Patient Characteristics at Diagnosis

Characteristic	IBC		Non-IBC		P [¶]
	No. of Pts	%	No. of Pts	%	
Total no. of Patients	25		57		
Age, years					
Mean (mini-max)	48.4(29–78)		52.5(24–80)		0.121
T at diagnosis (TNM)					
T4b-c	-		57	100	-
T4d	25	100	-		
Stage at diagnosis					
IIIB-IIIC	22	88	46	81	0.624
IV	3	12	11	19	
Supraclavicular nodes					
Yes	10	40	12	27	0.104
No	15	60	45	79	
Nuclear grade					
II	3	12	13	23	0.368
III	22	88	44	77	
Lymphatic invasion in BC					
Positive	19	76	18	32	
Negative	5	20	25	44	0.005
Unknown	1	4	14	25	
ER status [*]					
ER-positive	6	24	31	54	0.016
ER-negative	19	76	26	46	
HER2 status [*]					
HER2-amplified	12	48	17	30	0.136
HER2-normal	13	52	40	70	
Molecular subtype [†]					
Luminal A	4	16	13	23	<0.001

Characteristic	IBC		Non-IBC		P [¶]
	No. of Pts	%	No. of Pts	%	
Luminal B	1	4	9	16	
ERBB2 new positive	10	40	15	26	
Basal like	7	28	17	30	
Normal like	3	12	3	5	
Pathologic response [‡]					
pCR	6	24	4	7	
no-pCR	15	60	37	65	0.075
unknown (no surgery or lost f/u)	4	16	16	28	
Nodal status at surgery					
N0	6	24	15	26	
N 1-3	6	24	9	16	
N 4	10	40	18	32	0.795
Unknown	3	12	15	26	
Neoadjuvant chemotherapy [§]					
FAC or FEC or AC	2	8	6	11	
T/FAC or FEC or AC/ or Others	17	68	36	63	
T/FAC or FEC or AC/H/ or Others	6	24	7	12	0.579
Others	-		8	14	
Adjuvant endocrine therapy					
Yes/No	3/22		17/39		0.098
Event rate					
Alive/Dead	14/11		37/20		0.468

Abbreviations: Pts, patients; IBC, Inflammatory Breast Cancer; BC, Breast Cancer; ER, estrogen receptor; pCR, pathologic complete response.

* Receptor status was determined by gene expression.

[†] Molecular subtypes were identified by PAM50 prediction.

[‡] pCR refers to pathologic complete response both in breast and axilla.

[§] T, paclitaxel; F, 5-fluorouracil; A, doxorubicin; E, epirubicin; C, cyclophosphamide; H, trastuzumab.

[¶] P denotes results of significant test for the comparison between IBC vs Non-IBC by Fisher exact test or t-test.

Table 2

Class comparison test between IBC and Non-IBC in tumor subgroups

Significantly differentially expressed probe sets (n=18,940) with t-test									
No. of patients (IBC/Non-IBC)	ALL		ER+/HER2-		HER2+		ER-/HER2-		
	82 (25/57)	Global P value *	No. of Genes	Global P value *	No. of Genes	Global P value *	No. of Genes	Global P value *	
Parametric p value †									
0.05	1214	0.209	927	0.372	2065	0.051	989	0.279	
0.01	268	0.167	195	0.318	508	0.043	144	0.462	
0.005	130	0.177	90	0.388	265	0.039	68	0.458	
0.001	25	0.188	23	0.311	61	0.030	16	0.353	

Efron-Tibshirani's GSA test P-value* with prior defined gene sets									
No. of patients (IBC/Non-IBC) GeneList GeneSets	ALL		ER+/HER2-		HER2+		ER-/HER2-		
	82 (25/57)	Directions	P -value‡	Directions	P -value‡	Directions	P -value‡	Directions	
Wnt gene set ¹⁵	17	0.545	Non-IBC	0.375	IBC	0.415	Non-IBC	0.040	IBC
Laere genes ¹³	36	0.395	IBC	0.315	Non-IBC	0.330	Non-IBC	0.195	IBC
Bieche genes ⁴	54	0.245	IBC	0.015	IBC	0.180	Non-IBC	0.300	IBC
CD44 related signatures ¹⁶	55	0.135	Non-IBC	0.140	Non-IBC	0.055	Non-IBC	0.415	Non-IBC
Bertucci genes ¹⁴	71	0.230	Non-IBC	0.560	IBC	0.045	IBC	0.010	Non-IBC

IBC: Inflammatory Breast Cancer; ER: Estrogen Receptor.

* Global p value shows the probability of getting at least the same number of genes significant on a t-test (at the specified p level) by chance if there are no real differences between IBC and Non-IBC.

† The parametric p-value was derived from t-test.

‡ The Efron and Tibshirani gene set analysis method that employs “maxmean” statistics (under 1000 permutations).

Table 3

Gene sets overexpressed in IBC compared with Non-IBC

GO category	GO term	Number of genes	GSA test <i>P</i> value
Gene sets overexpressed in ER+/HER2-normal IBC compared with Non-IBC			
GO:0002675	Positive regulation of acute inflammatory response	11	0.001
GO:0002673	Regulation of acute inflammatory response	15	0.001
GO:0050729	Positive regulation of inflammatory response	31	0.001
GO:0050727	Regulation of inflammatory response	57	0.002
GO:0043407	Negative regulation of MAP kinase activity	40	0.003
GO:0031406	Carboxylic acid binding	17	0.005
GO:0045103	Intermediate filament-based process	25	0.005
GO:0010574	Regulation of vascular endothelial growth factor production	17	0.006
GO:0045104	Intermediate filament cytoskeleton organization	23	0.006
GO:0046928	Regulation of neurotransmitter secretion	12	0.007
GO:0033549	MAP kinase phosphatase activity	12	0.008
GO:0043500	Muscle adaptation	20	0.008
GO:0002526	Acute inflammatory response	97	0.008
GO:0014896	Muscle hypertrophy	15	0.009
GO:0042531	Positive regulation of tyrosine phosphorylation of STAT protein	22	0.009
GO:0051348	Negative regulation of transferase activity	92	0.009
GO:0033673	Negative regulation of kinase activity	86	0.010
Gene sets overexpressed in HER2-positive IBC compared with Non-IBC			
GO:0051668	Localization within membrane	15	0.001
GO:0051084	'de novo' posttranslational protein folding	15	0.001
GO:0009119	Ribonucleoside metabolic process	15	0.001
GO:0043681	Protein import into mitochondrion	16	0.001
GO:0009168	Purine ribonucleoside monophosphate biosynthetic process	22	0.001
GO:0006458	'de novo' protein folding	23	0.001
GO:0019206	Nucleoside kinase activity	23	0.001
GO:0042994	Cytoplasmic sequestering of transcription factor	11	0.002
GO:0006626	Protein targeting to mitochondrion	30	0.002
GO:0009116	Nucleoside metabolic process	41	0.002
GO:0003729	mRNA binding	90	0.002
GO:0043094	Cellular metabolic compound salvage	10	0.003
GO:0051220	Cytoplasmic sequestering of protein	14	0.003
GO:0022884	Macromolecule transmembrane transporter activity	15	0.003
GO:0042273	Ribosomal large subunit biogenesis	18	0.003
GO:0010466	Negative regulation of peptidase activity	21	0.003
GO:0005758	Mitochondrial intermembrane space	34	0.003
GO:0046128	Purine ribonucleoside metabolic process	13	0.004
GO:0019201	Nucleotide kinase activity	30	0.004
GO:0008344	Adult locomotory behavior	35	0.004

GO category	GO term	Number of genes	GSA test <i>P</i> value
GO:0019059	Initiation of viral infection	42	0.004
GO:0006446	Regulation of translational initiation	72	0.004
GO:0015935	Small ribosomal subunit	89	0.004
GO:0030159	Receptor signaling complex scaffold activity	24	0.005
GO:0031970	Organelle envelope lumen	41	0.005
GO:0046148	Pigment biosynthetic process	54	0.007
GO:0015934	Large ribosomal subunit	99	0.007
GO:0006144	Purine base metabolic process	24	0.008
GO:0009146	Purine nucleoside triphosphate catabolic process	13	0.009
GO:0042274	Ribosomal small subunit biogenesis	22	0.009
GO:0009161	Ribonucleoside monophosphate metabolic process	33	0.009
GO:0006195	Purine nucleotide catabolic process	20	0.010
GO:0030041	Actin filament polymerization	81	0.010
Gene sets overexpressed in ER-negative/HER2-normal IBC compared with Non-IBC			
GO:0035094	Response to nicotine	19	0.001
GO:0043279	Response to alkaloid	27	0.001
GO:0005548	Phospholipid transporter activity	35	0.001
GO:0048545	Response to steroid hormone stimulus	97	0.001
GO:0006800	Oxygen and reactive oxygen species metabolic process	78	0.002
GO:0002437	Inflammatory response to antigenic stimulus	14	0.003
GO:0005100	Rho GTPase activator activity	26	0.003
GO:0014902	Myotube differentiation	16	0.004
GO:0016528	Sarcoplasm	34	0.004
GO:0008367	Bacterial binding	12	0.005
GO:0014070	Response to organic cyclic substance	37	0.006
GO:0043627	Response to estrogen stimulus	62	0.006
GO:0002861	Regulation of inflammatory response to antigenic stimulus	13	0.007
GO:0048019	Receptor antagonist activity	14	0.008
GO:0016529	Sarcoplasmic reticulum	33	0.008
GO:0007631	Feeding behavior	41	0.008
GO:0002683	Negative regulation of immune system process	71	0.009
GO:0006656	Phosphatidylcholine biosynthetic process	13	0.010