Differentially Expressed Genes and Key Pathways in Triple Negative Breast Cancers: A Bioinformatics Analysis

Zuolin Shi
General Hospital of Northern Theatre command
Xiyu Liu
General Hospital of Northern Theatre command
Na Zhang
General Hospital of Northern Theatre command
Hongna Guan (hongnaguan@163.com)
General Hospital of Northern Theatre command https://orcid.org/0000-0002-5510-454X

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Abstract

Background: Triple negative breast cancer (TNBC) is usually associated with poor outcome, and is characterized by lack of estrogen and progesterone hormone receptors and lack of overexpression of HER2. The aim of this study was to identify gene signatures during TNBCs and uncover their potential mechanisms.

Methods: The gene expression profiles of GDS4069 were downloaded from GEO database. The GDS4069 dataset contained 19 samples, including 5 TNBCs, and 14 non-TNBCs (Control). The gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes pathway (KEGG) enrichment analyses were performed, and protein–protein interaction (PPI) network of the differentially expressed genes (DEGs) was constructed by Cytoscape software. The research was conducted according to the principles of the Declaration of Helsinki.

Results: In total, 603 DEGs were identified in TNBCs, including 199 up-regulated genes and 403 down-regulated genes. GO analysis results showed that up-regulated DEGs were significantly enriched in biological processes (BP), including adaptive immune response, immunological synapse, positive regulation of GTPase, positive regulation of c-Jun N-terminal kinase (JNK), and positive regulation of T cell, et al.; the down-regulated DEGs were significantly enriched in biological processes, including cilium assembly, protein transport, intracellular transport, cilium morphogenesis, anterior/posterior pattern specification, and positive regulation of cilium assembly. KEGG pathway analysis showed the up-regulated DEGs were enriched in hematopoietic cell lineage signaling pathway, cytokine-cytokine receptor interaction signaling pathway, and glycosphingolipid biosynthesis-globo series signaling pathway, while the down-regulated DEGs were enriched in peroxisome signaling pathway.

Conclusions: The present study indicated that the identified DEGs and hub genes promote our understanding of the molecular mechanisms underlying the development of TNBCs, and might be used as molecular targets and diagnostic biomarkers for the treatment of TNBCs.

Introduction

Historically, triple-negative breast cancer (TNBC) expresses low-level human epidermal growth factor receptor 2 (HER2), progesterone receptor (PR), and estrogen receptor (ER). TNBCs are often associated with aggressive biological behaviors and unfavorable prognosis. Unlike other kinds of breast cancers (ie, HER2-positive: Trastuzumab; hormone-receptor positive: endocrinotherapies), except for routine chemotherapy, TNBCs have no approved targeted-therapies until now[1]. Although the basic principles of diagnosis and management of TNBCs are similar to other subtypes of breast cancer, the chemotherapy sensitivity, pathologic and molecular characteristics, and risk factors are specific to TNBCs[2]. Up to now, the complex molecular biological mechanisms underlying the genesis and development of TNBCs remain little known, thus limiting TNBCs' treatments and preventions. So, further exploring the cellular mechanisms involved in molecular and pathologic characteristics of TNBCs and the genetic differences between TNBCs and non-TNBCs is of vital importance.

Microarrays, as the representative of high-throughput-platforms for analyzing genes' expressions, have been regarded as vital tools clinically: disease response prediction, new drug targets discovery, prognosis prediction, patient stratification, diseases' molecular classification, and molecular and cellular diagnoses, etc. There have already been several such studies, by virtue of microarray-technology, focusing on the DEGs, transduction pathways, molecular functions or biological processes of breast cancers[3, 4]. But little reliable molecular-biomarkers discriminating TNBC-tissues from non-TNBC-tissues have been detected.

In this research, from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov /geo/), the original data (GDS4069) was downloaded for a bioinformatics analysis[5]. Genetic expressions of breast-cancer cells in non-TNBC tissues were compared with those in TNBC tissues to find potential DEGs. Then we screened the DEGs by Gene-Spring software, followed by gene ontology (GO) and pathway enrichment-analysis. Through screening the DEGs and identifying the underlying biological pathways and functions, we could make the clearer clearest regarding TNBC's molecular biological mechanisms and gain more inspirations and enlightenments regarding TNBC's diagnosis, drug-targets and prognosis.

Methods

Microarray data

The genetic expression-profiles of GDS4069 were obtained from the GEO-database[6]. GDS4069, which was based on GPL6244 platform [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene) version], was submitted by Yang L, et al. in the journal of Oncogene(6). The GDS4069 dataset contained 19 samples, including 5 TNBCs and 14 non-TNBCs (control).

DEG Identifications

The TXT file (GPL6244 platform) constituted the main raw data. By adopting the software of Gene-Spring (version 11.5, Agilent, America), the raw data were firstly categorized into 2 subgroups: TNBCs and non-TNBCs, which was called a hierarchical clustering-analysis. with the same software, the principal component analysis (PCA) was used to conduct the probe quality-control. Probes whose intensity-values were less than 20% were filtered out. The classical t-test was used to detect DEGs whose changes were over two folds. $P < 0.01$ was set as the cutoff value of statistically significance.

Gene ontology and pathway enrichment analysis of DEGs

Gene-ontology analysis (GO) has been increasingly regarded as a valuable and helpful tool to annotate genes and gene-products or to identify distinctive molecular biological approaches and features for high-flux genomes and massive amounts of data of transcriptomics[7]. KEGG (http://www.gen ome.jp/), as

the bridge that leads from genomic data to higher-order functional data, is another important tool in bioinformatics researches on the functions of genes and gene-products[8]. In terms of gene linking, mapping users' genes to known, classical and related genetic annotations in DAVID (https://david.ncifcrf.gov/) plays pivotal roles in any high-throughput genetic function analyses[9]. For analyzing DEGs at functional levels, KEGG pathway analyses and GO enrichment analyses were conducted on DAVID (online version)[10]. $P < 0.01$ was set as the cutoff value of statistically significance.

Protein–protein interaction (PPI) module analysis

Search Tool for the Retrieval of Interacting Genes (STRING, version 10.0) is one of the commonest used online tools for evaluating protein–protein interactions (PPIs), which contains data of over 960,0000 proteins from over 2000 organisms[11]. The DEGs screened from the above procedures were mapped online to the STRING to evaluate the interactions among these DEGs (up-regulated and down-regulated). The mark of significance: 1) the combined score > 0.4; and 2) experimentally validated interactions[11]. By virtue of Cytoscape, the PPI network was drawn[12]. modules of PPI networks were screened on the plug-in Molecular Complex Detection (MCODE) in Cytoscape [The criteria: 1) number of nodes > 4; and 2) MCODE scores > 3][13]. Furthermore, for the DEGs in these modules, the pathway enrichment-analysis and functional analysis were further conducted. $P < 0.01$ was set as the cutoff value of statistically significance.

Results

Identification of DEGs

Five TNBC samples and 14 non-TNBC samples were included in this analysis. Using Gene-E software, series from all chips were separately analyzed, then the DEGs were eventually identified. On the basis of the Gene-E analysis results of GDS4069, 603 DEGs were finally identified; 403 were down-regulated and 199 were up-regulated. Figure 1 was the drawn heat map for DEG expressions (top 50 down-regulated and up-regulated genes).

GO term enrichment analysis

To identify over-represented GO-categories or KEGG-pathways, all DEGs were uploaded to DAVID online. We found that up-regulated DEGs were enriched in biological process significantly (BP), including adaptive immune response, immunological synapse, positive-regulation of GTPase, positive regulation of c-Jun N-terminal kinase (JNK), and positive-regulation of T cell, et al. (Table 1); the down-regulated DEGs enriched in biological process included cilium assembly, protein transport, intracellular transport, cilium morphogenesis, posterior/anterior pattern specifications, and positive-regulation of cilium assembly (Table 1). For molecular function (MF), the up-regulated DEGs were enriched in axon guidance receptor activity, actin binding, endopeptidase inhibitor activity, Ras guanyl-nucleotide exchange factor activity, ephrin receptor activity, and receptor activity; and the down-regulated DEGs were enriched in calcium ion binding, GTPase activity, Rab GTPase binding, RNA polymerase II repressing transcription factor binding, microtubule motor activity, calcium-dependent phospholipid binding (Table 1). Additionally, GO cell-component (CC) analyses also demonstrated that the up-regulated DEGs were significantly enriched in plasma membranes, external side of plasma membranes, integral component of plasma membranes, phagocytic vesicles, intracellular, and integral component of Golgi membranes, and down-regulated DEGs enriched in MKS complex, ciliary membrane, cytoplasmic vesicle membrane, ciliary basal body, trans-Golgi network, and BBSome (Table 1).

Table 1

Gene ontology analysis of differentially expressed genes associated with triple negative breast cancers.

Expression	Category	Term	Count	%	P-Value	Representative Genes	Fold Enrichment	FDR
Up-regulated	GOTERM_BP_DIRECT	GO:0007420~brain development	8	0.03845	7.62E-04	EPHA7, NES, EGR2, B3GNT5, SPHK1, PTCH1, CD27, HMX3	5.316027	1.175006
	GOTERM_BP_DIRECT	GO:0002250~adaptive immune response	7	0.033644	0.001115	BTLA, LAMP3, LILRA6, CD1C, CD79A, CLEC10A, CD7	5.97155	1.714521
	GOTERM_BP_DIRECT	GO:0001771~immunological synapse formation	3	0.014419	0.002648	CORO1A, CCL19, EPHB1	37.87669	4.026243
	GOTERM_BP_DIRECT	GO:0043547~positive regulation of GTPase activity	12	0.057676	0.005012	PLEKHG1, RGS21, RGS2, KL, RASGRP2, CCL19, RASGEF1C, IL5RA, SH3BP1, GFRA2, FGD4, GFRA3	2.681536	7.492278
	GOTERM_BP_DIRECT	GO:0046330~positive regulation of JNK cascade	4	0.019225	0.014551	CCR7, CCL19, FZD7, CD27	7.769578	20.32157
	GOTERM_BP_DIRECT	GO:2000525~positive regulation of T cell costimulation	2	0.009613	0.01566	EPHB6, CCR7	126.2556	21.70051
	GOTERM_CC_DIRECT	GO:0005886~plasma membrane	50	0.240315	2.55E-04	IL27RA, USH1G, OR4D10, FMNL1, RAB39B, TTC7B, FMN1, CCR7, CHRM3, CPAMD8, CABP2, PTCHD1	1.613952	0.300248
	GOTERM_CC_DIRECT	GO:0005887~integral component of plasma membrane	19	0.09132	0.01802	LTK, IL27RA, KL, SELL, CD1C, SLC26A10, RHAG, EPHB1, KCNMB2, EPHB6, EPHA7, BPI, CHRM3	1.78616	19.31002
	GOTERM_CC_DIRECT	GO:0009897~external side of plasma membrane	6	0.028838	0.021866	CCR7, SELL, CD79A, IGKC, CD27, GFRA3	3.747096	22.96144
	GOTERM_CC_DIRECT	GO:0045335~phagocytic vesicle	3	0.014419	0.034221	FMNL1, RAB7B, CORO1A	10.23245	33.6906
	GOTERM_CC_DIRECT	GO:0005622~intracellular	17	0.081707	0.039793	RAB7B, LTK, RAB39B, KL, NCALD, CCL19, RASGEF1C, COTL1, FAM49A, CCR7, B3GNT5, PLCG2	1.697727	38.06718
	GOTERM_CC_DIRECT	GO:0030173~integral component of Golgi membrane	3	0.014419	0.06971	ST3GAL2, ST8SIA1, QSOX2	6.880443	57.36862
	GOTERM_MF_DIRECT	GO:0008046~axon guidance receptor activity	3	0.014419	0.001133	EPHA7, EPHB1, GFRA3	56.96625	1.473848

Expression	Category	Term	Count	%	P-Value	Representative Genes	Fold Enrichment	FDR
	GOTERM_MF_DIRECT	GO:0003779~actin binding	7	0.033644	0.018031	FMN1, CORO1A, NCALD, TBC1D21, COTL1, FGD4, ALKBH4	3.346938	21.21163
	GOTERM_MF_DIRECT	GO:0004866~endopeptidase inhibitor activity	3	0.014419	0.035847	CPAMD8, CST7, ITIH2	9.969094	38.01635
	GOTERM_MF_DIRECT	GO:0005088~Ras guanyl-nucleotide exchange factor activity	4	0.019225	0.054897	KL, IL5RA, GFRA2, GFRA3	4.623348	52.27804
	GOTERM_MF_DIRECT	GO:0005003~ephrin receptor activity	2	0.009613	0.07913	EPHB6, EPHB1	24.1675	66.04406
	GOTERM_MF_DIRECT	GO:0004872~receptor activity	5	0.024032	0.079567	EPHB6, CHRM3, IL10RB, GFRA2, CD7	3.062702	66.25449
Down-regulated	GOTERM_BP_DIRECT	GO:0042384~cilium assembly	9	0.017984	0.001001	TMEM67, BBS4, MKS1, BBS5, TTC30A, TTC8, TCTN2, RAB3IP, DNAH5	4.415849	1.637692
	GOTERM_BP_DIRECT	GO:0015031~protein transport	15	0.029974	0.005767	BBS4, BBS5, GOLT1A, CHMP5, SNX7, TTC8, CHMP2B, C15ORF38-AP3S2, HOOK2, ARF3, AAGAB, RAB11A	2.310402	9.098846
	GOTERM_BP_DIRECT	GO:0046907~intracellular transport	4	0.007993	0.006817	BBS4, BBS5, C14ORF79, APPBP2	10.1401	10.67053
	GOTERM_BP_DIRECT	GO:0060271~cilium morphogenesis	8	0.015986	0.007126	TMEM67, BBS4, MKS1, BBS5, TCTN1, TTC8, TCTN2, DNAH5	3.578858	11.12755
	GOTERM_BP_DIRECT	GO:0009952~anterior/posterior pattern specification	6	0.011989	0.010068	HOXB3, HOXC6, RARG, HOXC5, HOXB6, CELSR1	4.563043	15.37288
	GOTERM_BP_DIRECT	GO:0045724~positive regulation of cilium assembly	3	0.005995	0.011026	BBS4, TAPT1, CCP110	18.25217	16.7141
	GOTERM_CC_DIRECT	GO:0036038~MKS complex	4	0.007993	7.81E-04	TMEM67, MKS1, TCTN1, TCTN2	20.94713	1.036368
	GOTERM_CC_DIRECT	GO:0060170~ciliary membrane	6	0.011989	8.62E-04	TMEM67, BBS4, BBS5, TTC8, TCTN2, SCNN1A	8.022304	1.143178
	GOTERM_CC_DIRECT	GO:0030659~cytoplasmic vesicle membrane	9	0.017984	9.07E-04	TMEM67, GPRC5C, AP1M2, SNX7, RAB11A, RAB13, SFN, APPBP2, RAB21	4.48867	1.202744
	GOTERM_CC_DIRECT	GO:0036064~ciliary basal body	8	0.015986	9.49E-04	BBS4, TAPT1, MKS1, BBS5, RAB28, BBOF1, TTC8, RAB3IP	5.129909	1.257525

Expression	Category	Term	Count	%	P-Value	Representative Genes	Fold Enrichment	FDR
	GOTERM_CC_DIRECT	GO:0005802~trans-Golgi network	8	0.015986	0.006029	SLC11A2, BECN1, RAB11A, RAB38, RAB13, SYT17, RAB21, ATP7B	3.696552	7.747883
	GOTERM_CC_DIRECT	GO:0034464~BBSome	3	0.005995	0.008382	BBS4, BBS5, TTC8	20.94713	10.61837
	GOTERM_MF_DIRECT	GO:0005509~calcium ion binding	23	0.04596	0.002853	REPS2, ENPP1, EFCAB6, TBC1D9, CELSR1, DNAH7, P4HTM, ANXA2, EFHD1, EFHC1, CLGN, PRRG4	1.990847	3.95574
	GOTERM_MF_DIRECT	GO:0003924~GTPase activity	11	0.021981	0.004665	MFN2, GPN3, ADSSL1, RAB28, ARF3, GNA11, RAB11A, RAB38, RAB13, RAB21, TUBB4B	2.917468	6.392294
	GOTERM_MF_DIRECT	GO:0017137~Rab GTPase binding	8	0.015986	0.005927	TBC1D8, MLPH, TBC1D9, SYTL4, SYTL2, RGP1, KIF16B, ANXA2	3.705224	8.054846
	GOTERM_MF_DIRECT	GO:0001103~RNA polymerase II repressing transcription factor binding	4	0.007993	0.009002	BBS4, BBS5, SIN3A, TTC8	9.194444	11.9928
	GOTERM_MF_DIRECT	GO:0003777~microtubule motor activity	6	0.011989	0.00929	BBS4, KIF16B, APPBP2, DNAH7, DYNC1I2, DNAH5	4.654688	12.35306
	GOTERM_MF_DIRECT	GO:0005544~calcium-dependent phospholipid binding	5	0.009991	0.01399	ANXA9, SYTL4, SYTL2, SYT17, ANXA2	5.350216	18.04773

KEGG pathway analysis

Through KEGG analyses, **Supplemental Table S1** showed the most evident enriched pathways in which the up- or down-regulated DEGs were involved in. The up-regulated DEGs were enriched in hematopoietic cell lineage signaling-pathway (Figure 2A), the signaling-pathway of cytokine-receptor interactions (Figure 2B), and glycosphingolipid biosynthesis-globo series signaling-pathway. Down-regulated DEGs were enriched in peroxisome signaling pathway.

Module-screening from PPI networks

According to the STRING-database, the top 10 hub-nodes with high degrees were explored. The hub-genes included MYB proto-oncogene, transcription factor (MYB), charged multivesicular body protein 2B (CHMP2B), phospholipase C gamma 2 (PLCG2), lysine demethylase 8 (KDM8), C-C motif chemokine receptor 7 (CCR7), CD27 molecule (CD27), male-specific lethal 3 homolog (Drosophila) (MSL3), H2A histone family member V (H2AFV), Bardet-Biedl syndrome 5 (BBS5), and selectin L (SELL). The total number of nodes was 74. The total number of edges was 17. The average node degree was 0.459. The clustering coefficient was 0.953. The PPI enrichment p-value was 0.0189. Moreover, with plug-ins MCODE, all edges and nodes were analyzed. The most significant module was selected, and the function-annotation of the involved genes was analyzed (**Supplemental Table S2**, Figure 3). Enrichment-analysis displayed that the genes in this module, in terms of biological process, was mainly related to positive-regulations of immunity system processes (Pathway ID: GO.0002684), positive-regulation of neutrophil chemotaxis (Pathway ID: GO.0090023), and negative-regulation of leukocyte apoptosis processes (Pathway ID: GO.2000107). In terms of cellular component analysis, these hub genes were significantly involved in external side of plasma membrane (Pathway ID: GO: 0009897).

Discussion

TNBCs account for around 20% of breast cancers across the world (approximately 0.2 million cases every year) [14, 15]. Compared with hormone positive breast cancers, TNBCs are more common in young women (<40 year-old)[15]. Risk factors associated with the diagnosis of TNBCs include: race (African-American women)[16], premenopausal status[16], maternal-related factors (nulliparity and breastfeeding are associated with a lower risk[17]; obesity and younger age at first pregnancy are related to increased risks[15, 17]. By definition, TNBC lacks immunohistochemical (IHC) expressions of the ER, PR, and HER2. As the 3 biomarkers represent the only known approved target-therapy, more comprehensively and deeply learning other biological factors promoting TNBCs' development increasingly became a hot field. Because the high-throughput sequencings and microarrays could simultaneously measure thousands of genes' expression status, these technologies have been adopted widely to provide valuble information about potential therapeutic-targets of TNBCs. Herein, we identified 403 down-regulated and 199 up-regulated DEGs between non-TNBC and TNBC cell tissues through bioinformatics-analysis[6]. Function-annotation demonstrated that these DEGs mainly contributed to the positive-regulation of GTPase, positive-regulation of JNK, cilium assembly, protein transport, and cilium morphogenesis, et al.

From the GEO dataset (GDS4069), we extracted gene-expression data of 5 TNBC samples and 14 non-TNBC samples [6]. All samples were extract using the Agilent microarrays. Comparing gene expression levels between early TNBCs and non-TNBCs, the investigators identified 199 up-regulated and 403 down-regulated genes in early TNBCs[6]. The up-regulated genes were associated with axon guidance receptor activity, endopeptidase inhibitor activity, Ras guanyl-nucleotide exchange factor activity, ephrin receptor activity. The down-regulated genes were involved in the microtubule motor activity, RNA polymerase-II repressing transcription-factor binding, and calcium-dependent phospholipid binding, et al. For better understanding the DEG interactions, KEGG pathway and GO analyses were also conducted.

The GO-analysis suggested that the up-regulated DEGs were mainly enriched in adaptive immunological responses, immunological synapse, positive regulation of GTPase, positive regulation of c-Jun N-terminal kinase (JNK), and positive regulation of T cell, et al.; and down-regulated DEGs were significantly enriched in biological processes, including cilium assembly, protein transport, intracellular transport, cilium morphogenesis, anterior/posterior pattern specification, and positive regulation of cilium assembly, which is in consistence with the previous finding that GTPase activating protein Git2 is a main cause for breast cancer initiation and metastatic colonization[18]. JNKs are associated with skeleton proteins JNK-interacting proteins and their upstream kinases: JNKK2 and JNKK1 after the activation. Mediated by activation-protein 1 like GM-CSF IL-8 and RANTES, JNKs are involved in cytokine productions, inflammatory conditions, cell proliferation and differentiation, apoptosis and neurodegeneration[19]. Shen et al. demonstrated that it was through the ROS/JNK/ATF-2/Bcl-2 signaling-pathway that cambogin functions as a proapoptotic agent, a potential future treatment against breast cancer[20].

Moreover, the KEGG-pathways of the up-regulated DEGs included hematopoietic cell lineage signaling pathway (Figure 2A), the signaling-pathway of cytokine-cytokine receptor interactions (Figure 2B), and glycosphingolipid biosynthesis-globo series signaling pathway[21]. The hematopoietic cell kinase (HCK) is a main kinase involved in hematopoietic cell lineage. Excessive hematopoietic cell lineage pathway activations are related to several leukemia subtypes and enhance cells' survival and proliferations and by physical associations with oncogenic-fusion-proteins, and with function-interactions with receptor tyrosine-kinases. Increased activities of hematopoietic-cell lineage are related to decreased patient survival-rates, and are also popular in lots of other solid tumors, like colon and breast cancers[22]. Cytokine-to-cytokine receptor interaction signaling-pathway detected in the present analysis was also found in Scheiber et al.'s study. Global expression analysis and RNA-Seq data from invasive breast cancer cell lines showed alterations in a few cellular signaling-pathways related to breast cancers, including the cytokine-to-cytokine receptor interaction signaling-pathway[23]. Down-regulated DEGs were associated with Peroxisome signaling-pathway. The peroxisome proliferator-activated receptor (PPAR) is a ligand-activated transcription-factor, which belongs to the nuclear hormone receptor superfamily. Chandran et al. found that PPARα expressions increased significantly in breast cancer tissues, implicating that stimulating PPARα by clinically approved, well-tolerated, and safe clofibrate might serve as a more effective and safer method for targeting the signaling, inflammatory, and lipogenic pathways in aggressive breast cancers[24].

PPI networks with DEGs were also constructed and the top degree hub-genes were listed: MYB, CHMP2B, PLCG2, KDM8, CCR7, CD27, MSL3, H2AFV, BBS5 and SELL. MYB gene, having been regarded as an oncogene, could encode a kind of protein with 3 DNA-binding domains serving as a transcription-regulator, which was essential in hematopoiesis-regulations, and might be aberrently rearranged or expressed or undergo translocation in the lymphomas and leukemia. Drabsch et al. found that shRNA-mediated MYB knockdown could initiate breast cancer cell's differentiations, and significantly sensitise them to pro-apoptotic and differentiative effects of differentiation-inducing agents[25].

Another hub gene is CCR7, located in 17q21.2. Its encoded protein, belonging to the G-protein-coupled receptor family, has been regarded as a gene induced by the Epstein-Barr virus (EBV), and one of the mediators of EBV effects on B-lymphocytes. CCR7s could activate T or B lymphocytes and are expressed in lots of lymphoid-tissues, which have been found to be able to control migrations of memory-T-cells to inflamed-tissues, as well as stimulate dendritic-cell maturations. Chemokine-receptors, for instance, CCR7, interact with and their ligands in both primary and metastatic breast cancer cells, which is pivotal for malignant disseminations and progressions[26]. CCR7 overexpression also occurs in gastric cancers, esophageal cancers, and lung cancers with lymph-node metastasis. Cabioglu et al. also found that CCR7 was a new bio-marker that was able to predict lymph-node metastasis in breast cancers[27]. Together with the present finding, CCR7 in TNBCs may play critical roles in TNBCs' tendency to metastasis and the patients' poor prognosis.

Module analyses of the PPI-network demonstrated that developments of TNBCs were correlated to several pathways. For GO.0002684, the pathways' function is positively regulating immune system process. Many data suggest that breast cancer is immunogenic. Immunosurveillance is critical for breast cancer. The proof that breast cancer may elicit an immune response are: 1) genetic instability leading to increased number of mutations, translated into

more neoantigens; 2) the prognostic value of an immunity-related gene signature; and 3) the presence of infiltrating immune cells and lymphocytes (TILs) within the tumor microenvironment. These characteristics are more pronounced in TNBCs and HER-2-positive tumor, therefore they are considered the most immunogenic breast cancer subtypes[28]. TNBC has been divided in six molecular subtypes, including an immunomodulatory subtype characterized by an immune gene expression pattern, high number of immune cells infiltrating the tumor stroma, higher programmed death-ligand 1 (PD-L1) expressions and high mutation rates. Thus, the signaling pathway of positive-regulation of immunologic system processes represent promising candidates for pharmacologic evaluations and for therapeutically treating TNBCs.

In short, the present study provided a comprehensive bioinformatics-analysis of DEGs, which might be involved in TNBC progresses. This study provided a set of useful targets for future investigations into the molecular and cellular mechanisms and bio-markers. However, deeper molecular biological experiments are needed to confirm the functions of the identified genes in breast cancers and TNBCs.

Declarations

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Conflict of interests

All authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Authors' Contributions

Zuolin Shi, Na Zhang and Xiyu Liu performed writing the manuscript and analyzed and interpreted the data. Hongna Guan was major contributors in study design. All authors read and approved the final manuscript.

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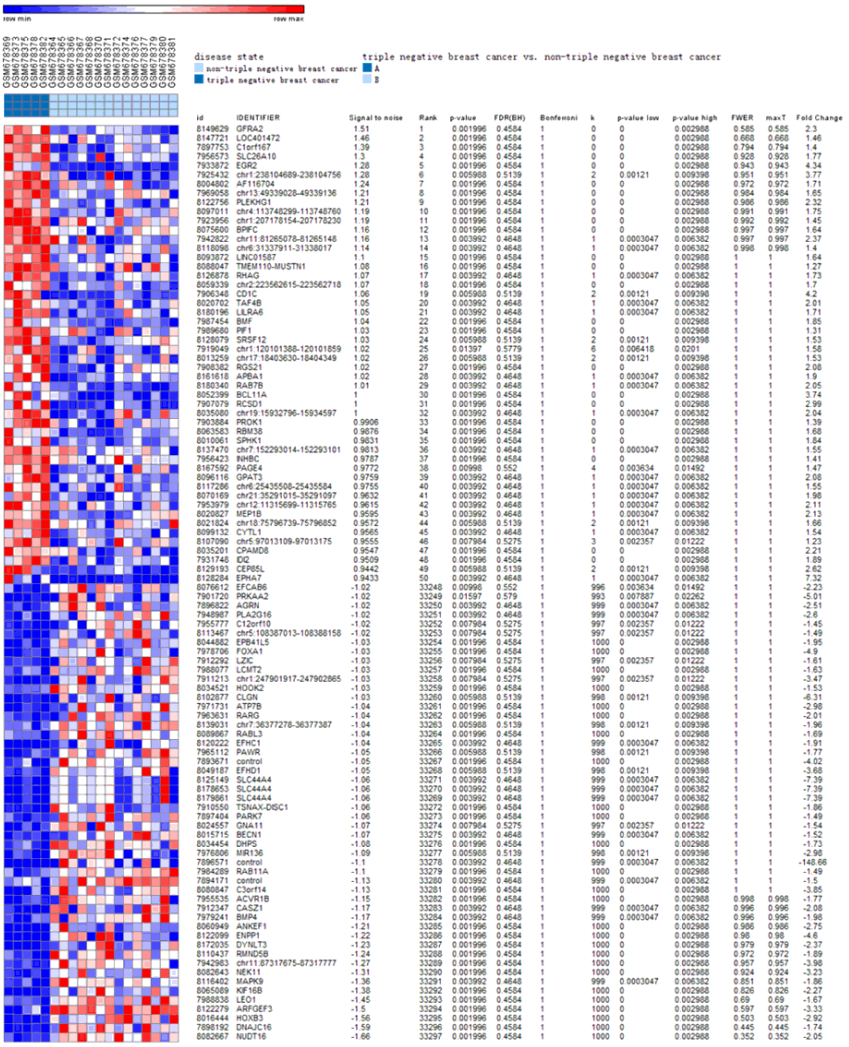
Figures



id	IDENTIFIER	Signal to noise	Rank	p-value	FDR(BH)	Bonferroni	k	p-value low	p-value high	FWER	maxT	Fold Change
8149629	GFRA2	1.51	1	0.001996	0.4584	1	0	0	0.002988	0.585	0.585	2.3
8147721	LOC401472	1.46	2	0.001996	0.4584	1	0	0	0.002988	0.668	0.668	1.46
7897753	C1orf167	1.39	3	0.001996	0.4584	1	0	0	0.002988	0.794	0.794	1.4
7956573	SLC26A10	1.3	4	0.001996	0.4584	1	0	0	0.002988	0.928	0.928	1.77
7933872	EGR2	1.28	5	0.001996	0.4584	1	0	0	0.002988	0.943	0.943	4.34
7925432	chr1:238104689-238104756	1.28	6	0.005988	0.5139	1	2	0.00121	0.009398	0.951	0.951	3.77
8004802	AF116704	1.24	7	0.001996	0.4584	1	0	0	0.002988	0.972	0.972	1.71
7969058	chr13:49339028-49339136	1.21	8	0.001996	0.4584	1	0	0	0.002988	0.984	0.984	1.65
8122756	PLEKHG1	1.21	9	0.001996	0.4584	1	0	0	0.002988	0.986	0.986	2.32
8097011	chr4:113748299-113748760	1.19	10	0.001996	0.4584	1	0	0	0.002988	0.991	0.991	1.75
7923956	chr1:207178154-207178230	1.19	11	0.001996	0.4584	1	0	0	0.002988	0.992	0.992	1.45
8075600	BPIFC	1.16	12	0.001996	0.4584	1	0	0	0.002988	0.997	0.997	1.64
7942822	chr11:81265078-81265148	1.16	13	0.003992	0.4648	1	1	0.0003047	0.006382	0.997	0.997	2.37
8118098	chr6:31337911-31338017	1.14	14	0.003992	0.4648	1	1	0.0003047	0.006382	0.998	0.998	1.4
8093872	LINC01587	1.1	15	0.001996	0.4584	1	0	0	0.002988	1	1	1.64
8088047	TMEM110-MUSTN1	1.08	16	0.001996	0.4584	1	0	0	0.002988	1	1	1.27
8126878	RHAG	1.07	17	0.003992	0.4648	1	1	0.0003047	0.006382	1	1	1.73
8059339	chr2:223562615-223562718	1.07	18	0.001996	0.4584	1	0	0	0.002988	1	1	1.7
7906348	CD1C	1.06	19	0.005988	0.5139	1	2	0.00121	0.009398	1	1	4.2
8020702	TAF4B	1.05	20	0.003992	0.4648	1	1	0.0003047	0.006382	1	1	2.01
8180196	LILRA6	1.05	21	0.003992	0.4648	1	1	0.0003047	0.006382	1	1	1.71
7987454	BMF	1.04	22	0.001996	0.4584	1	0	0	0.002988	1	1	1.85
7969680	PIF1	1.03	23	0.001996	0.4584	1	0	0	0.002988	1	1	1.31
8128079	SRSF12	1.03	24	0.005988	0.5139	1	2	0.00121	0.009398	1	1	1.53
7919049	chr1:120101388-120101859	1.02	25	0.01397	0.5779	1	6	0.006418	0.0201	1	1	1.58
8013259	chr17:18403630-18404349	1.02	26	0.005988	0.5139	1	2	0.00121	0.009398	1	1	1.53
7908382	BOLS21	1.02	27	0.001996	0.4584	1	0	0	0.002988	1	1	2.08
8161618	APBA1	1.02	28	0.003992	0.4648	1	1	0.0003047	0.006382	1	1	1.9
8180340	RAB7B	1.01	29	0.003992	0.4648	1	1	0.0003047	0.006382	1	1	2.05
8052399	BCL11A	1	30	0.001996	0.4584	1	0	0	0.002988	1	1	3.74
7907079	RCSD1	1	31	0.001996	0.4584	1	0	0	0.002988	1	1	2.99
8035080	chr19:15932796-15934597	1	32	0.003992	0.4648	1	1	0.0003047	0.006382	1	1	2.04
7903884	PROK1	0.9906	33	0.001996	0.4584	1	0	0	0.002988	1	1	1.39
8063583	RBM38	0.9876	34	0.001996	0.4584	1	0	0	0.002988	1	1	1.68
8010061	SPHK1	0.9831	35	0.001996	0.4584	1	0	0	0.002988	1	1	1.84
8137470	chr7:152293014-152293101	0.9813	36	0.003992	0.4648	1	1	0.0003047	0.006382	1	1	1.55
7956423	NHBC	0.9787	37	0.001996	0.4584	1	0	0	0.002988	1	1	1.41
8167592	PAGE4	0.9772	38	0.00998	0.552	1	4	0.003634	0.01492	1	1	1.47
8096116	GPAT3	0.9759	39	0.003992	0.4648	1	1	0.0003047	0.006382	1	1	2.08
8117286	chr6:25435508-25435584	0.9755	40	0.003992	0.4648	1	1	0.0003047	0.006382	1	1	1.55
8079169	chr21:35291015-35291097	0.9632	41	0.003992	0.4648	1	1	0.0003047	0.006382	1	1	1.98
7953979	chr12:11315699-11315765	0.9615	42	0.003992	0.4648	1	1	0.0003047	0.006382	1	1	2.11
8020827	MEP1B	0.9595	43	0.003992	0.4648	1	1	0.0003047	0.006382	1	1	2.13
8021824	chr18:75796739-75796852	0.9572	44	0.005988	0.5139	1	2	0.00121	0.009398	1	1	1.66
8099132	CYTL1	0.9565	45	0.003992	0.4648	1	1	0.0003047	0.006382	1	1	1.54
8107090	chr5:97013109-97013175	0.9555	46	0.007984	0.5275	1	3	0.002357	0.01222	1	1	1.23
8035201	CPAMD8	0.9547	47	0.001996	0.4584	1	0	0	0.002988	1	1	2.21
7931748	DIO2	0.9509	48	0.001996	0.4584	1	0	0	0.002988	1	1	1.89
8129193	CEP85L	0.9442	49	0.005988	0.5139	1	2	0.00121	0.009398	1	1	2.62
8128284	EPHA7	0.9433	50	0.003992	0.4648	1	1	0.0003047	0.006382	1	1	7.32
8076612	EFCAB6	-1.02	33248	0.00998	0.552	1	996	0.003634	0.01492	1	1	-2.23
7901720	PRKAA2	-1.02	33249	0.01597	0.579	1	993	0.007887	0.02262	1	1	-5.01
7896822	AGRN	-1.02	33250	0.003992	0.4648	1	999	0.0003047	0.006382	1	1	-2.51
7948987	PLA2G16	-1.02	33251	0.003992	0.4648	1	999	0.0003047	0.006382	1	1	-2.6
7955777	C12orf10	-1.02	33252	0.007984	0.5275	1	997	0.002357	0.01222	1	1	-1.45
8113467	chr5:108387013-108388158	-1.02	33253	0.007984	0.5275	1	997	0.002357	0.01222	1	1	-1.49
8044882	EPB41L5	-1.03	33254	0.001996	0.4584	1	1000	0	0.002988	1	1	-1.95
7978706	FOXA1	-1.03	33255	0.001996	0.4584	1	1000	0	0.002988	1	1	-4.9
7912992	LZK	-1.03	33256	0.007984	0.5275	1	997	0.002357	0.01222	1	1	-1.61
7988077	LCMT2	-1.03	33257	0.001996	0.4584	1	1000	0	0.002988	1	1	-1.63
7911213	chr1:247901917-247902865	-1.03	33258	0.007984	0.5275	1	997	0.002357	0.01222	1	1	-3.47
8034521	HOOK2	-1.03	33259	0.001996	0.4584	1	1000	0	0.002988	1	1	-1.53
8102877	CLGN	-1.03	33260	0.005988	0.5139	1	998	0.00121	0.009398	1	1	-8.31
7971731	ATP7B	-1.04	33261	0.001996	0.4584	1	1000	0	0.002988	1	1	-2.98
7963631	RARG	-1.04	33262	0.001996	0.4584	1	1000	0	0.002988	1	1	-2.01
8139031	chr7:36377278-36377387	-1.04	33263	0.005988	0.5139	1	998	0.00121	0.009398	1	1	-1.96
8089867	RABL3	-1.04	33264	0.001996	0.4584	1	1000	0	0.002988	1	1	-1.69
8120222	EFHC1	-1.04	33265	0.003992	0.4648	1	999	0.0003047	0.006382	1	1	-1.91
7965112	PAWR	-1.05	33266	0.005988	0.5139	1	998	0.00121	0.009398	1	1	-1.77
7893671	control	-1.05	33267	0.001996	0.4584	1	1000	0	0.002988	1	1	-4.02
8049187	EFHD1	-1.05	33268	0.005988	0.5139	1	998	0.00121	0.009398	1	1	-3.68
8125149	SLC44A4	-1.06	33271	0.003992	0.4648	1	999	0.0003047	0.006382	1	1	-7.39
8178653	SLC44A4	-1.06	33270	0.003992	0.4648	1	999	0.0003047	0.006382	1	1	-7.39
8179861	SLC44A4	-1.06	33269	0.003992	0.4648	1	999	0.0003047	0.006382	1	1	-7.39
7910550	TSNAX-DISC1	-1.06	33272	0.001996	0.4584	1	1000	0	0.002988	1	1	-1.86
7897404	PABK7	-1.06	33273	0.001996	0.4584	1	1000	0	0.002988	1	1	-1.49
8024557	DNA11	-1.07	33274	0.007984	0.5275	1	997	0.002357	0.01222	1	1	-1.54
8015715	BECN1	-1.07	33275	0.003992	0.4648	1	999	0.0003047	0.006382	1	1	-1.52
8034454	DHPS	-1.08	33276	0.001996	0.4584	1	1000	0	0.002988	1	1	-1.73
7976806	MIR136	-1.09	33277	0.005988	0.5139	1	998	0.00121	0.009398	1	1	-2.98
7896571	control	-1.1	33278	0.003992	0.4648	1	999	0.0003047	0.006382	1	1	-148.66
7964289	RAB11A	-1.1	33279	0.001996	0.4584	1	1000	0	0.002988	1	1	-1.49
7894171	control	-1.13	33280	0.003992	0.4648	1	999	0.0003047	0.006382	1	1	-1.5
8080847	C3orf14	-1.13	33281	0.001996	0.4584	1	1000	0	0.002988	1	1	-3.85
7955535	ACVR1B	-1.15	33282	0.001996	0.4584	1	1000	0	0.002988	0.998	0.998	-1.77
7912347	CASZ1	-1.17	33283	0.003992	0.4648	1	999	0.0003047	0.006382	0.996	0.996	-2.08
7979241	BMP4	-1.17	33284	0.003992	0.4648	1	999	0.0003047	0.006382	0.996	0.996	-1.98
8060949	ANKEF1	-1.21	33285	0.001996	0.4584	1	1000	0	0.002988	0.986	0.986	-2.75
8122099	ENPP1	-1.22	33286	0.001996	0.4584	1	1000	0	0.002988	0.98	0.98	-4.6
8172035	DYNLT3	-1.23	33287	0.001996	0.4584	1	1000	0	0.002988	0.979	0.979	-2.37
8110437	RMND5B	-1.24	33288	0.001996	0.4584	1	1000	0	0.002988	0.972	0.972	-1.89
7942983	chr11:87317675-87317777	-1.27	33289	0.001996	0.4584	1	1000	0	0.002988	0.957	0.957	-3.98
8082643	NEK11	-1.31	33290	0.001996	0.4584	1	1000	0	0.002988	0.924	0.924	-3.23
8116402	MAPK9	-1.36	33291	0.003992	0.4648	1	999	0.0003047	0.006382	0.851	0.851	-1.86
8065089	KIF16B	-1.38	33292	0.001996	0.4584	1	1000	0	0.002988	0.826	0.826	-2.27
7988836	LEO1	-1.45	33293	0.001996	0.4584	1	1000	0	0.002988	0.69	0.69	-1.67
8122279	ARFGEF3	-1.5	33294	0.001996	0.4584	1	1000	0	0.002988	0.597	0.597	-3.33
8016444	HOXB3	-1.56	33295	0.001996	0.4584	1	1000	0	0.002988	0.503	0.503	-2.92
7898192	DNAJC16	-1.59	33296	0.001996	0.4584	1	1000	0	0.002988	0.445	0.445	-1.74
8082667	NUDT16	-1.66	33297	0.001996	0.4584	1	1000	0	0.002988	0.352	0.352	-2.05

Figure 1

Heat map of the top 100 differentially expressed genes (50 up-regulated genes and 50 down-regulated genes). Red: up-regulation; purple: down-regulation.

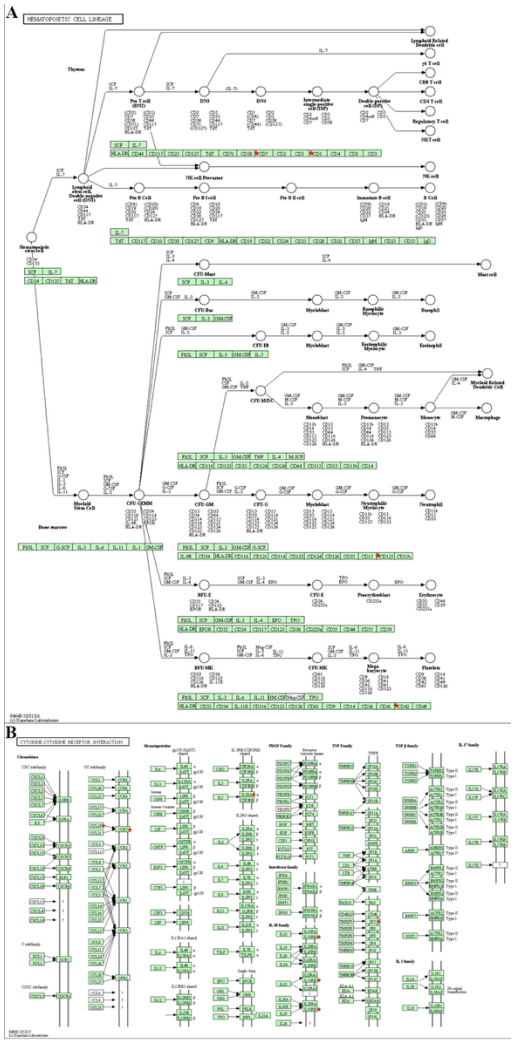


Figure 2

Related signaling pathways. **A**. Hematopoietic cell lineage signaling pathway sketch map. The genes marked by red pentagram are the deferentially expressed genes screened out via GO analysis. **B**. Cytokine-cytokine receptor interaction signaling pathway signaling pathway sketch map. The genes marked by red pentagram are the deferentially expressed genes screened out via GO analysis.

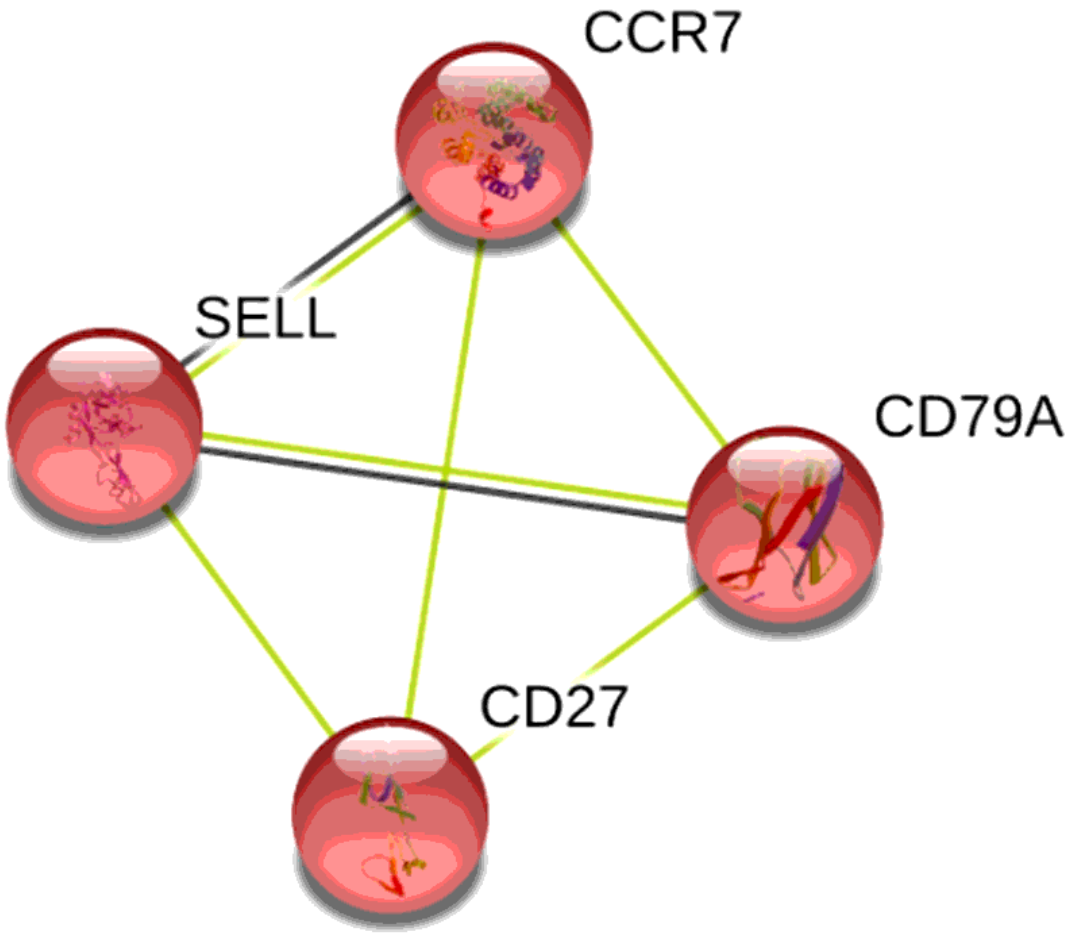


Figure 3

A significant module from the protein–protein interaction (PPI) network.

Supplementary Files

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