Original Article

Identification of key genes involved in tamoxifen-resistant breast cancer using bioinformatics analysis

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Background: The purpose of the present study was to investigate the molecular mechanisms of tamoxifen resistance in breast cancer and to identify potential targets for antitamoxifen resistance.

Methods: Differentially expressed genes (DEGs) in tamoxifen-resistant and tamoxifen-sensitive breast cancer cells were assessed using the GSE67916 dataset acquired from the Gene Expression Omnibus database. Gene ontology (GO) and pathway enrichment analyses were applied to investigate the functions and pathways of the DEGs. Subsequently, the protein-protein interaction (PPI) network was constructed using the Search Tool for the Retrieval of Interacting Genes (STRING), and subnetworks were further analyzed by Molecular Complex Detection (MCODE). The PPI network and subnetworks were visualized using Cytoscape software.

Results: In total, 438 DEGs were identified, of which 300 were upregulated and 138 were downregulated. The DEGs were significantly enriched in the protein binding, cellular response to estradiol stimulus, and immune response GO terms while the most significant pathways included the mitogen-activated protein kinase (MAPK) signaling pathway in cancer. The PPI network of DEGs was constructed with 288 nodes and 629 edges, and 2 subnetworks were screened out from the entire network.

Conclusions: A number of significant hub DEGs were identified based on their degree of connectivity in the PPI network, , included *MAPK1* (node degree 36), *ESR1* (node degree 27), *SMARCA4* (node degree 27), *RANBP2* (node degree 25), and *PRKCA* (node degree 21). These critical hub genes were found to be related to tamoxifen resistance in breast cancer. The results of this study further the understanding of tamoxifen resistance at the molecular level and identify potential therapeutic targets for tamoxifen-resistant breast cancer.

Keywords: Tamoxifen resistance; breast cancer; differentially expressed genes (DEGs); protein-protein interaction (PPI) network; bioinformatics analysis

Submitted Jul 17, 2021. Accepted for publication Nov 03, 2021.
doi: 10.21037/tcr-21-1276
View this article at: https://dx.doi.org/10.21037/tcr-21-1276

Introduction

The incidence of breast cancer is increasing in China and in most other countries (1). Breast cancer is the most common malignant neoplasm and the primary cause of cancer-related death in women in the United States (2,3). According to the literature, approximately 70% to 80% of breast cancers express estrogen receptor alpha (ERα) (4,5). Endocrine therapies have significantly reduced the mortality and recurrence rates of ER-positive

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breast cancer patients. Tamoxifen, a selective estrogen receptor modulator (SERM), has proven effective in the treatment of ER-positive breast cancer in premenopausal women (6). A meta-analysis confirmed that tamoxifen was able to reduce the recurrence rate of breast cancer by approximately 37% and the mortality rate by 29% in women <45 years of age (7); however, about 50% of ER-positive breast cancers present with an inherent resistance to endocrine therapy, and 30% to 40 % of those reactive to tamoxifen will become resistant to tamoxifen (3). The mechanisms of tamoxifen resistance are related to many factors, such as the activation of oncogenes, the inactivation of antioncogenes, changes in the expression of ERα, alterations in co-regulatory proteins, and the influence of growth factor signal pathways (8). Nevertheless, the phenomenon of tamoxifen resistance is still a major clinical problem in breast cancer therapy.

With the application of bioinformatics technology to microarray analysis, significant progress has been made in the study of tamoxifen resistance mechanisms. Some genes have been found to be associated with mechanisms of tamoxifen resistance; for example, the amplified in breast cancer 1 (*AIB1*) gene, also known as steroid receptor coactivator (SRC-3), is overexpressed in more than 50% of breast cancers (8). *AIB1* overexpression correlates with the recurrence of breast tumors, shorter disease-free survival times, and poorer overall survival (9). Evidence has been provided to show that *AIB1* contributes to tamoxifen resistance (10,11). Moreover, a knockdown of *AIB1* level in the tamoxifen-resistant breast cancer cell line BT47 was shown to restore the sensitivity of breast cancer cells to tamoxifen (9). Anterior gradient 2 (AGR2), a secretory protein, is a member of the protein disulfide isomerase (PDI) family. It has been discovered that tamoxifen may stimulate the expression of AGR2, and AGR2 overexpression plays a role in tamoxifen resistance (12). Human epidermal growth factor receptor 2 (HER2) and G protein-coupled estrogen receptor 1 (GPER) have been demonstrated to contribute to tamoxifen resistance (10,13). Additionally, the mechanisms of tamoxifen resistance are also related to significant pathways, such as the human epidermal growth factor receptor 2 (HER2) tyrosine kinase pathway and the phosphatidylinositol 3-kinase (PI3K) cell survival pathway (8). Progress has been achieved in illustrating the mechanisms of tamoxifen resistance. Nevertheless, the current knowledge on tamoxifen resistance remains inadequate.

In the present study, a bioinformatics method was applied to analyze gene expression profiles in tamoxifen-resistant breast cancer and to identify the differences between differentially expressed genes (DEGs) in tamoxifen-resistant and tamoxifen-sensitive breast cancer cells. A protein-protein interaction (PPI) network of DEGs was structured for discovering the potential and crucial genes involved in tamoxifen-resistant breast cancer. The purpose of the study was to strengthen the understanding of the mechanisms of tamoxifen resistance and to identify potential novel therapeutic targets for tamoxifen-resistant breast cancer. We present the following article in accordance with the STREGA reporting checklist (available at https://dx.doi.org/10.21037/tcr-21-1276).

Methods

Gene expression data analysis

Based on the GPL570 platform data (Affymetrix Human Genome U133 Plus 2.0 Array; Affymetrix, Inc., Santa Clara, CA, USA), as reported by Elias *et al.* (14), the GSE67916 gene expression profiles were downloaded from the Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo). These data included 4 tamoxifen-resistant MCF7/S0.5 cell lines, TamR1, TamR4, TamR7, and TamR8, and 1 tamoxifen-sensitive MCF7/S0.5 cell line. In total, 18 samples consisting of 9 tamoxifen-resistant breast cancer cell samples and 9 tamoxifen-sensitive breast cancer cell samples were analyzed in the present study.

Statistical analysis

The data were processed and analyzed using R v. 3.6.3 statistical software (R Foundation for Statistical Computing, Vienna, Austria). The average expression value of the probes mapped to the same gene was regarded as the final expression value for the gene.

Data processing and DEG analysis

The DEGs in the tamoxifen-resistant breast cancer cell line and the tamoxifen-sensitive cell line were analyzed using the Linear Models for Microarray Analysis (limma) v. 3.48.3 software package (https://www.bioconductor.org/). Absolute value of log fold change >1.5 and adjusted P<0.01 were considered to be the threshold values for DEGs.

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Gene ontology (GO) and pathway enrichment analysis

GO_produced by Gene Ontology Consortium, aims to conveniently and accurately represent the requests for updates to biological information received from scientists making gene and protein annotations (15). In addition, it classifies relevant gene sets into their respective pathways using the Kyoto Encyclopedia of Genes and Genomes (KEGG; https://www.genome.jp/kegg/) database.

To analyze the differentially expressed genes, the GO functions and KEGG pathway enrichment analyses were investigated using the Database for Annotation, Visualization and Integration Discovery (DAVID) v. 6.8 software (https://david.abcc.ncifcrf.gov/). GO categories, including molecular function (MF), biological process (BP), and cellular component (CC), were applied to the analysis of the classification of the DEGs. A P value of <0.05 was set as the cutoff criterion.

Construction of the PPI network

The PPI network was established for the DEGs employing the Search Tool for the Retrieval of Interacting Genes (STRING) v. 11 (https://string-db.org), an online database that gathers integrated information of PPIs (16). The interactions of protein pairs in the STRING database were displayed using a combined score, and a combined score of >0.4 was established as the cutoff value in the network. The PPI network was subsequently presented using the Cytoscape v. 3.5.0 software platform, and the hub genes were screened out according to their degree of connectivity in the PPI network (expressed as node degree, referring to number of neighbors). The subnetworks with a node degree >10 were appraised using the Molecular Complex Detection (MCODE) plugin in Cytoscape (17). Subsequently, the subnetwork functions were analyzed by GO and KEGG pathway enrichment analyses using DAVID.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Result

Identification of DEGs

A total of 438 DEGs were identified in the 9 tamoxifen-resistant breast cancer cell samples and the 9 tamoxifen-sensitive breast cancer cell samples using the limma package, of which 300 genes were upregulated and 138 genes were downregulated (*Table 1*).

GO function enrichment analyses of upregulated and downregulated DEGs

GO functional analyses were carried out on the upregulated and downregulated DEGs, respectively. The top 5 GO terms identified in the 3 GO categories (BP, CC, and MF) are shown in *Table 1*. The GO terms significantly involved in the GO categories of upregulated DEGs were cytoplasm, cytosol, and perinuclear region of cytoplasm in the CC category; protein binding, poly(A) RNA binding, and transcription factor binding in the MF category; and protein transport, negative regulation of transcription from RNA polymerase II promoter, and transcription, DNA-templated in the BP category. The GO terms significantly involved in the GO categories of downregulated DEGs were plasma membrane and extracellular exosome in the CC category; protein binding, kinase activity, and cadherin binding involved in cell-cell adhesion in the MF category; and cellular response to estradiol stimulus, positive regulation of transcription, DNA template, and interferon gamma–mediated signaling pathway in the BP category.

KEGG pathway enrichment analyses of upregulated and downregulated DEGs

The upregulated DEGs were significantly enriched in the leukocyte transendothelial migration, lysosome, and RIG-I-like receptor signaling pathways. The downregulated DEGs were significantly enriched in the viral carcinogenesis pathways, the signaling pathways regulating the pluripotency of stem cells, and the HIF-1 signaling pathway (*Table 2*).

PPI network analysis

Based on the STRING database analysis, a total of 629 protein pairs with combined scores of >0.4 were identified. As demonstrated in *Figure 1*, the PPI network consisted of 288 nodes and 629 edges. The nodes of *MAPK1* (node degree 36), *ESR1* (node degree 27), *SMARCA4* (node degree 27), *RANBP2* (node degree 25), and *PRKCA* (node degree 21) were hub proteins in the PPI network.

Two subnetworks (subnetworks 1 and 2) with >10 nodes were discerned using the MCODE plugin (*Figure 2*). The hub proteins *MAPK1* and *ESR1* were demonstrated to be involved in subnetwork 1. Subnetwork 1 was primarily associated with the following GO terms: protein binding, DNA binding, nucleoplasm, immune response, positive

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Table 1 Identification of DEGs between tamoxifen resistant and tamoxifen sensitive samples

	Genes
Upregulated	*PRKCA, HHLA3, PGM2L1, C8orf44-SGK3///SGK3, C6orf89, MR1, FBP1, SCYL2, CSGALNACT1, FAS, ZDHHC5, MTHFD2, SDC2, EFNA5, CEP97, ENC1, ELOVL2-AS1, EIF2AK2, LMBRD2, RERG, MAP1B, CYB5B, ARHGEF6, SH3BGRL, NUDT7, YPEL2, CLIC3, CLIC4, RHOBTB3, NEK2, USP47, TPBG, SMARCA4, P2RX4, CHRNA5, MYO1B, ZFP36L2, CAB39, POLI, C5orf24, TIA1, KCNJ8, USP42, TMEM87B, GOPC, ZGRF1, KCNK5, ACVR1C, KCTD1, FBXO38, DDX3X, GPATCH2, C12orf65, ASAH1, SQSTM1, NME7, ENTPD3, CTDSPL2, ACTA2, MIR612///NEAT1, BTN3A2, CTSO, CTNNA1, TRIM38, DGKH, SPTAN1, OSMR, NKTR, BBX, CDK13, GLUD2, TEX9, FARSA, RAB32, TMC5, KIAA0513, ONECUT2, BHLHE41, SLC1A4, SELM, MBNL1, HNRNPD, WDR90, SPAG9, LOC101928524, SETBP1, LMNB1, TMPRSS3, AGFG1, PARP9, LOC642852, TPR, GALC, VAV3, HIST1H2AM, NR2F2, CPEB4, FOXO3B///FOXO3, CYP4B1, HIST1H2AE, GADD45B, RAB5A, TRIM6-TRIM34///TRIM34, ARHGAP5, ARID1A, ID2B///ID2, SLC7A8, JUND, RBAK, MINCR, NR1D2, HLA-DQB1, AKAP1, SGMS2, IGF1R, FAM76A, PIK3R1, RECQL4, FUS, DLX2, MIA3, LOC101060835///LOC100996809///HLA-DRB5///HLA-DRB4///HLA-DRB1///HLA-DQB1, CAPN8, FAM117A, FDFT1, F11R, ZNF431, MT1F, NUP210, ASAP1, TM7SF2, SP110, SDC1, SYTL2, TFPI, ST8SIA4, SALL4, SNX27, GALNT2, CDKL5, KIF5C, DCAF16, SNORA28///EIF5, GNB4, FREM2, LGALS3BP, ABCD3, ZNF850, EXPH5, EHD1, TMX1, ATP6V1A, LIPA, GBP2, MAP2, DGKA, OGDH, ADGRL2, TRIO, STIP1, NFIB, MAP3K1, PCDH19, PMEPA1, FAM213A, MAN1A1, AMOTL1, HIST2H4B///HIST4H4///HIST2H4A///HIST1H4L///HIST1H4E///HIST1H4B///HIST1H4H///HIST1H4C///HIST1H4J///HIST1H4K///HIST1H4F///HIST1H4D///HIST1H4A///HIST1H4I, C1orf115, TIAM1, FNIP1, ZYX, RBM22, GATC, CLCC1, HSPA2, U2SURP, SP100, LOC100288911, MVD, GDPD1, MAVS, SMTN, PHF10, C17orf80, EDEM3, LYST, PPP2R3B, SLC24A3, PAPOLA, ADCY1, RANBP2, DACH1, TFPI2, BCAT1, HDAC8, ZNF253, MYCBP2, TRIM59///IFT80, GGCX, PGGT1B, HMGN5, ANXA9, SLC22A15, LOC101929597///TBCD, HIPK1, KPNA1, NBPF1, CFL1, GSAP, WTAP, TPP1, GBP3, MYEF2, DENND4C, FNIP2, SCUBE2, KRIT1, CCDC83, GULP1, 6-Mar, SUGT1, FYTTD1, CLDN3, NDRG1, STX17, EFNB2, C21orf91, KDM4B, C14orf132, ATXN1, CENPF, MSMO1, LY6E, ACYP1, AKAP13, ERVMER34-1, AGA, ANLN, EIF5A2, GOSR2, KLHL20, ANOS1, RBM48, R3HCC1L, STC2, CITED1, GGT6, SECISBP2L, NDST4, EXOC6, RUNDC1, USP10, JPX, USP34, BTN3A2///BTN3A3, GEM, SRSF4, SLC4A7, BBS9, ITGB4, XPO7, AGR2, TANK, IRF7, CITED2, CACNG4, CYP1B1, PRNP, NR3C1, KRT15, SERPINI1, MAPK1IP1L, PHF23, CEBPG, GRN, ZMYM2, METTL8, CA5BP1, DNMT3A, TANC2, MPPED2, HERPUD2, PABPN1, TBL1X, TMEFF2, TBRG1, RAB30, ZNF711, SEC61A1, LAMP1, SYNRG, BRINP3, ZYG11B, MBTPS1, SRRT, ZNRF2, SEPHS1, FBXW11, PHLDA1*
Downregulated	*SHANK2, CPSF6, MOCS3, C1GALT1, TOB2, WBSCR22, AP1S3, WLS, METTL4, DEGS1, MED30, MAPKAP1, HLA-A, RBM26, NCOA3, SLC39A6, SLC9A7, CD163L1, EPHA4, GFPT1, SNORD3D///SNORD3C///SNORD3B-2///SNORD3A///SNORD3B-1, BFSP1, KIF3A, TFRC, PRKAB1, ARHGEF28, UBE2J1, PLA2G16, ZNF652, SLC18B1, IFNGR1, CCDC88C, RAD52, FARSB, CAPRIN1, FRMD5, CSNK1A1, MIR1908///FADS1, MRPL30, SETD5, SCPEP1, TIMP2, FAM83A, ATP1B1, LZTFL1, ZNF236, LOC101060835///HLA-DQB1, COL12A1, CA8, LINC00839, CHTF18, GPX3, PSME4, MBP, GPR87, HIST1H4H, PM20D2, SERPINA1, PSEN1, KIFAP3, SYTL4, FGFR2, ERAP1, SGK1, LSS, SOX2, FSIP1, TM9SF1, PCYOX1, TFF1, IRF9, ARFIP1, ZFP90, RAP2A, RBMS1, LARP4, LOC101930578///TPTE2P2, RAMP1, ESR1, MPZL2, KBTBD2, ZNFX1, ELL2, SEPT2, SLC44A1, S100A4, EPC1, MYB, COBLL1, HHIPL2, B4GALT1, HSPA4, FLVCR2, SLC12A6, CA12, LOC100505984///ITGB6, SCN1B, JPH1, LNPEP, SOWAHC, KCTD6, PPM1E, PAPSS2, ATP10D, CDC14B, TBC1D3P1-DHX40P1///RNFT1, PJA1, ZNF703, JMY, MAPK1, FAM189A2, SHMT2, CD59, ARSG, HIST1H2BD, MIR4784///MZT2A///MZT2B, MGP, DLC1, STK38, CPNE4, SOX3, C1orf226, SPANXA2///SPANXA1, TMEM192, HOOK3, CXCL12, PGR, PIK3R3, MALRD1, SMAD5, WWP1, IL20, GBP1, ELOVL2, GFRA1, PLXDC2, GREB1, FCMR*

438 DEGs indentified between tamoxifen resistant and tamoxifen sensitive samples are listed, of which 300 upregulated and 138 downregulated. DEGs, differentially expressed genes.

regulation of transcription, and DNA template. Pathway analysis showed enrichment of the viral carcinogenesis and cancer pathways (*Table 3*). In contrast, subnetwork 2 was associated with the following GO terms: nucleus, extracellular exosome, and the binding with protein and DNA (*Table 4*). The most significant pathway in subnetwork 2 was the MAPK signaling pathway (*Table 4*).

Discussion

Tamoxifen acts as a first-line therapy in the treatment of ER-positive breast cancer for premenopausal women. Yet, the phenomenon of tamoxifen resistance has become a major clinical problem in breast cancer therapy. It is therefore critical that novel therapeutic targets for tamoxifen-resistant breast cancer are discovered. To date,

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Table 2 GO and pathway enrichment analyses for DEGs

Category	Term	Count	P value
Upregulated DEGs			
GOTERM_CC_DIRECT	GO:0005737-cytoplasm	98	4.79×10^{-3}
GOTERM_CC_DIRECT	GO:0005829-cytosol	69	2.19×10^{-3}
GOTERM_CC_DIRECT	GO:0016020-membrane	54	2.05×10^{-4}
GOTERM_CC_DIRECT	GO:0048471-perinuclear region of cytoplasm	19	5.18×10^{-3}
GOTERM_CC_DIRECT	GO:0043231-intracellular membrane-bounded organelle	17	8.95×10^{-3}
GOTERM_MF_DIRECT	GO:0005515-protein binding	159	1.69×10^{-3}
GOTERM_MF_DIRECT	GO:0005524-ATP binding	34	2.04×10^{-2}
GOTERM_MF_DIRECT	GO:0044822-poly(A) RNA binding	26	3.98×10^{-2}
GOTERM_MF_DIRECT	GO:0000166-nucleotide binding	13	7.41×10^{-3}
GOTERM_MF_DIRECT	GO:0008134-transcription factor binding	11	1.22×10^{-2}
GOTERM_BP_DIRECT	GO:0000122-negative regulation of transcription from RNA polymerase II promoter	20	1.45×10^{-2}
GOTERM_BP_DIRECT	GO:0045892-negative regulation of transcription, DNA-templated	15	2.12×10^{-2}
GOTERM_BP_DIRECT	GO:0015031-protein transport	12	4.04×10^{-2}
GOTERM_BP_DIRECT	GO:0007399-nervous system development	10	3.23×10^{-2}
GOTERM_BP_DIRECT	GO:0051260-protein homooligomerization	8	1.89×10^{-2}
KEGG_PATHWAY	hsa05164:Influenza A	11	6.44×10^{-4}
KEGG_PATHWAY	hsa05161:Hepatitis B	7	3.48×10^{-2}
KEGG_PATHWAY	hsa04670:Leukocyte transendothelial migration	7	1.41×10^{-2}
KEGG_PATHWAY	hsa04142:Lysosome	7	1.58×10^{-2}
KEGG_PATHWAY	hsa04622:RIG-I-like receptor signaling pathway	5	2.96×10^{-2}
Downregulated DEGs			
GOTERM_CC_DIRECT	GO:0005886-plasma membrane	37	4.59×10^{-2}
GOTERM_CC_DIRECT	GO:0070062-extracellular exosome	36	1.36×10^{-4}
GOTERM_CC_DIRECT	GO:0005829-cytosol	31	4.64×10^{-2}
GOTERM_CC_DIRECT	GO:0005654-nucleoplasm	28	2.77×10^{-2}
GOTERM_CC_DIRECT	GO:0016020-membrane	25	1.04×10^{-2}
GOTERM_MF_DIRECT	GO:0005515-protein binding	80	1.03×10^{-3}
GOTERM_MF_DIRECT	GO:0005524-ATP binding	15	1.67×10^{-2}
GOTERM_MF_DIRECT	GO:0042802-identical protein binding	12	1.67×10^{-2}
GOTERM_MF_DIRECT	GO:0016301-kinase activity	5	9.09×10^{-3}
GOTERM_MF_DIRECT	GO:0098641-cadherin binding involved in cell-cell adhesion	4	3.35×10^{-2}
GOTERM_BP_DIRECT	GO:0045893-positive regulation of transcription, DNA-templated	10	1.10×10^{-2}
GOTERM_BP_DIRECT	GO:0060333-interferon-gamma-mediated signaling pathway	4	1.39×10^{-2}

Table 2 (*continued*)

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Table 2 (*continued*)

Category	Term	Count	P value
GOTERM_BP_DIRECT	GO:0006730-one-carbon metabolic process	3	1.90×10^{-2}
GOTERM_BP_DIRECT	GO:0071392-cellular response to estradiol stimulus	3	2.03×10^{-2}
GOTERM_BP_DIRECT	GO:0060348-bone development	3	3.57×10^{-2}
KEGG_PATHWAY	hsa04919:Thyroid hormone signaling pathway	6	2.56×10^{-3}
KEGG_PATHWAY	hsa05203:Viral carcinogenesis	6	2.81×10^{-2}
KEGG_PATHWAY	hsa04550:Signaling pathways regulating pluripotency of stem cells	5	2.95×10^{-2}
KEGG_PATHWAY	hsa04960:Aldosterone-regulated sodium reabsorption	4	4.14×10^{-3}
KEGG_PATHWAY	hsa04066:HIF-1 signaling pathway	4	4.87×10^{-2}

Top five GO terms in diversetables and major pathways are listed. Count, number of DEGs; GO,gene ontology; DEGs, differentially expressed genes; MF, molecular function; BP, biological process; CC, cellular component; KEGG, KyotoEncyclopedia of Genes and Genomes.

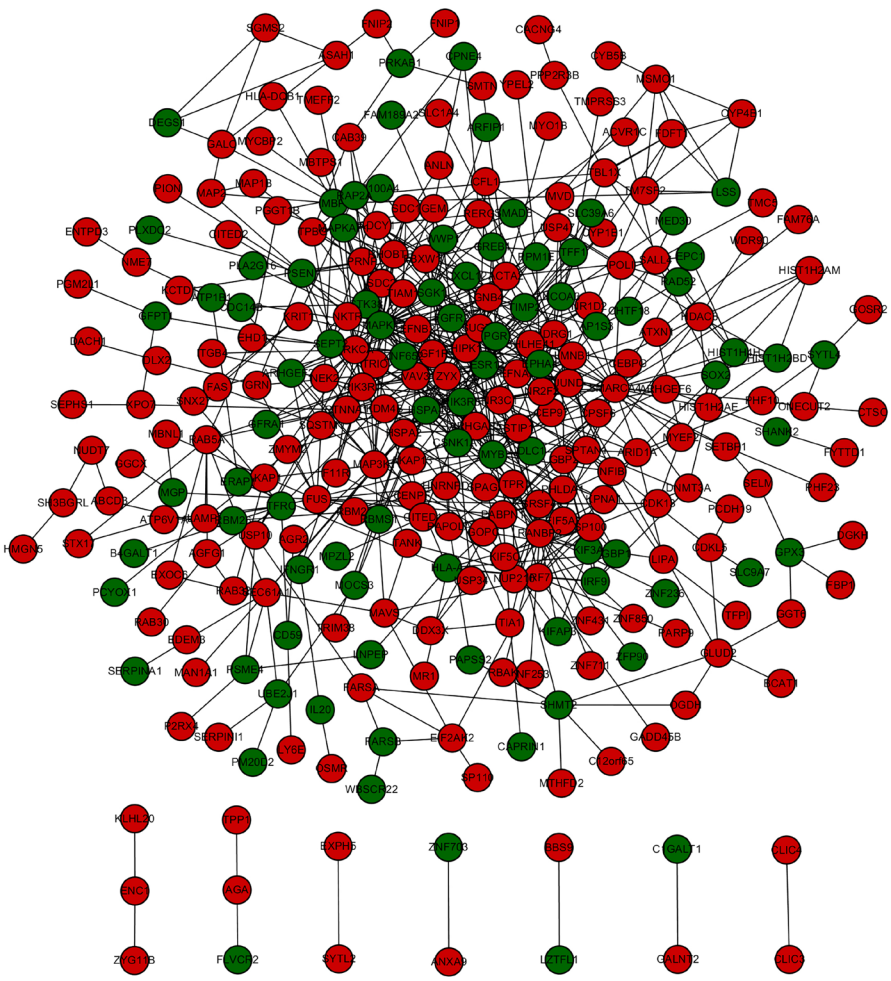

Figure 1 Construction of the PPI network. Identification of hub proteins in the PPI network, among 288 nodes and 629 edges, using the STRING database analysis. PPI, protein-protein interaction; STRING, Search Tool for the Retrieval of Interacting Genes.

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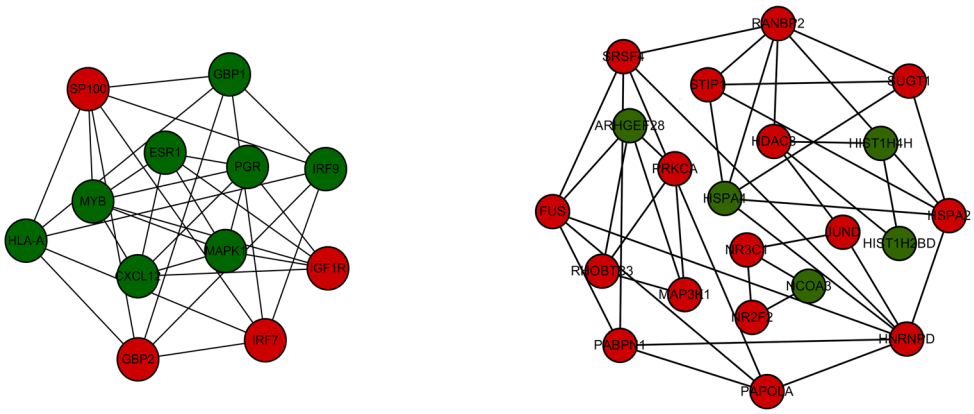

Figure 2 Construction of 2 subnetworks in the PPI network. Differentiation of the subnetworks with 10 more nodes using the MCODE plugin. PPI, protein-protein interaction; MCODE, Molecular Complex Detection.

Table 3 GO and pathway analysis for genes in subnetwork 1

Category	Term	Count	P value
GOTERM_CC_DIRECT	GO:0005654-nucleoplasm	6	1.71×10^{-2}
GOTERM_CC_DIRECT	GO:0000139-Golgi membrane	3	4.76×10^{-2}
GOTERM_MF_DIRECT	GO:0005515-protein binding	11	8.43×10^{-3}
GOTERM_MF_DIRECT	GO:0003677-DNA binding	6	2.64×10^{-3}
GOTERM_MF_DIRECT	GO:0042802-identical protein binding	5	9.88×10^{-4}
GOTERM_MF_DIRECT	GO:0019899-enzyme binding	3	1.89×10^{-2}
GOTERM_MF_DIRECT	GO:0001077-transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding	3	9.85×10^{-3}
GOTERM_BP_DIRECT	GO:0060333-interferon-gamma-mediated signaling pathway	6	5.30×10^{-10}
GOTERM_BP_DIRECT	GO:0060337-type I interferon signaling pathway	5	6.21×10^{-8}
GOTERM_BP_DIRECT	GO:0045893-positive regulation of transcription, DNA-templated	5	2.43×10^{-4}
GOTERM_BP_DIRECT	GO:0006955-immune response	4	2.22×10^{-3}
GOTERM_BP_DIRECT	GO:0006366-transcription from RNA polymerase II promoter	4	3.89×10^{-3}
KEGG_PATHWAY	hsa05203:Viral carcinogenesis	5	8.43×10^{-5}
KEGG_PATHWAY	hsa05168:Herpes simplex infection	4	1.36×10^{-3}
KEGG_PATHWAY	hsa04914:Progesterone-mediated oocyte maturation	3	5.33×10^{-3}
KEGG_PATHWAY	hsa04114:Oocyte meiosis	3	8.26×10^{-3}
KEGG_PATHWAY	hsa05200:Pathways in cancer	3	8.91×10^{-3}

Top five GO terms in diversetables and major pathways are listed. Count, number of DEGs; GO,gene ontology; DEGs, differentially expressed genes; MF, molecular function; BP, biological process; CC, cellular component; KEGG, KyotoEncyclopedia of Genes and Genomes.

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Table 4 GO and pathway analysis for genes in subnetwork 2

Category	Term	Count	P value
GOTERM_CC_DIRECT	GO:0005634-nucleus	17	3.84×10^{-5}
GOTERM_CC_DIRECT	GO:0005654-nucleoplasm	11	7.49×10^{-2}
GOTERM_CC_DIRECT	GO:0070062-extracellular exosome	10	3.58×10^{-3}
GOTERM_CC_DIRECT	GO:0000790-nuclear chromatin	4	1.55×10^{-3}
GOTERM_CC_DIRECT	GO:0000786-nucleosome	4	1.91×10^{-4}
GOTERM_MF_DIRECT	GO:0005515-protein binding	20	8.13×10^{-4}
GOTERM_MF_DIRECT	GO:0003723-RNA binding	7	5.39×10^{-5}
GOTERM_MF_DIRECT	GO:0003677-DNA binding	7	1.75×10^{-2}
GOTERM_MF_DIRECT	GO:0008270-zinc ion binding	6	1.54×10^{-2}
GOTERM_MF_DIRECT	GO:0044822-poly(A) RNA binding	6	1.34×10^{-2}
GOTERM_MF_DIRECT	GO:0019899-enzyme binding	4	8.87×10^{-3}
GOTERM_BP_DIRECT	GO:0000398-mRNA splicing, via spliceosome	5	1.80×10^{-4}
GOTERM_BP_DIRECT	GO:0031124-mRNA 3'-end processing	3	1.93×10^{-3}
GOTERM_BP_DIRECT	GO:0006369-termination of RNA polymerase II transcription	3	3.14×10^{-3}
KEGG_PATHWAY	hsa05034:Alcoholism	5	9.61×10^{-4}
KEGG_PATHWAY	hsa05322:Systemic lupus erythematosus	4	4.70×10^{-3}
KEGG_PATHWAY	hsa04010:MAPK signaling pathway	4	2.69×10^{-2}

Top five GO terms in diversetables and major pathways are listed. Count, number of DEGs; GO, geneontology; MF, molecular function; BP, biological process; CC, cellular component; KEGG, Kyoto Encyclopedia of Genes and Genomes.

many relevant proteins and pathways have been identified. The receptor tyrosine kinase (RTK) family and activation of the phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of the rapamycin (mTOR) receptor pathway are thought to be important mechanisms of tamoxifen resistance (18). In recent years, it has been proven that low expression of ERα36 increases the tamoxifen sensitivity of breast cancer cells by blocking the epidermal growth factor receptor/extracellular signal-regulated kinase (EGFR/ERK) signaling pathway (19). Gao *et al.* found that the LEM4 structural protein inhibits the sensitivity of breast cancer cells to tamoxifen by accelerating the G1 to S phase (G1/S) transition (20). In addition, it is reported that highly expressed cell division cycle–associated protein 8 (CDCA8) may increase tamoxifen resistance in breast cancer cells (21). Elias *et al.* (14) reported that several functional genes, including *FYN*, *PRKCA*, *ITPR1*, *DPYD*, *DACH1*, *LYN*, *GBP1*, and *PRLR*, are related to a reduction in tamoxifen sensitivity. In the present study, we reanalyzed the updated data. A total of 438 DEGs in tamoxifen-resistant and tamoxifen-sensitive breast cancer cell-lines were identified, including 300 upregulated and 138 downregulated genes. GO function and KEGG pathway enrichment analysis were used to analyze the DEGs, which were associated with protein binding and immune response. In addition, signaling pathway analysis revealed that these DEGs were mainly involved in the MAPK signaling pathway in cancer. Subsequently, the PPI network and subnetworks were constructed in order to explore the interactions of the DEGs. The top t nodes (by maximum node degree, referring to number of neighbors) were identified, including *MAPK1*, *ESR1*, *SMARCA4*, *RANBP2*, and *PRKCA*.

MAPK1, also known as ERK, encodes the protein which is involved in a wide variety of cellular processes, such as proliferation, differentiation, transcription regulation, and development (22,23).With the phosphorylation of 90 kDa ribosomal S6 kinases (p90RSK2), ERK mediates mitogen-induced proliferation signals from the cell membrane to the nucleus (24). *MAPK1* mutations are associated with many human cancers, such as breast cancer, prostate

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cancer, and ovarian cancer (23,25,26). Furthermore, some studies have proven that *MAPK1* hyperactivation plays a role in tamoxifen resistance (27,28). ERα is phosphorylated by increased ERK activity, which leads to a ligand-independent transcription of ERα and an agonistic activity of tamoxifen (29). The present study indicated that *MAPK1* was a hub protein with a node degree score of 36 in the PPI network. Therefore, the *MAPK1* gene may be a crucial regulator in tamoxifen-resistant breast cancer.

SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 4 (*SMARCA4*), also known as brahma-related gene-1 (*BRG1*), codes a core protein of the SWI/SNF chromatin-remodeling complex, which impacts chromatin and the transcription of target genes via the energy of ATP hydrolysis. Moreover, it also controls many cellular processes, such as DNA repair (30). BRG1 mutations are found in lung cancer and Burkitt's lymphoma (31,32). High BRG1 expression has been associated with poor survival and cell proliferation of triple-negative breast cancer (TNBC) (33). Furthermore, prior reports have indicated that BRG1 is related to estrogen receptor and is recruited to estrogen-responsive promoters (34). Therefore, *BRG1* may play a role in the mechanism of estrogen antagonists. Nacht *et al.* demonstrated the function of *BRG1* in hormone-dependent gene repression in breast cancer cells. *BRG1* plays a key role in hormone-dependent cell proliferation and apoptosis (35). In addition, *SMARCA4* was observed acting as a potential regulator of differentially expressed proteins in male breast cancer (36); however, the linkage between *BRG1* and tamoxifen resistance is rarely reported. A later mechanistic study revealed that *SOX4* and *SMARCA4* cooperatively regulate PI3K/Akt signaling and lead to the genesis and/or progression of TNBC (37). In our study, the *SMARCA4* gene was elevated in the tamoxifen-resistant samples and was a hub protein in the PPI network. Thus, *SMARCA4* may be a potential target in the treatment of tamoxifen-resistant breast cancer.

The *ESR1* gene has been found to encode the ERα and a ligand-dependent transcription factor of the nuclear receptor family (38). ERα can regulate the activities of genes in various biological and tumor progression processes, and plays a key role in endocrine therapies for ER-positive breast cancer (39). Significant research efforts have demonstrated that the loss of ERα expression or function may contribute to resistance to tamoxifen therapy (8). The ligand-independent activity of ERα mutants may mediate resistance to tamoxifen (38,40). In accordance with the findings of the present study, decreased *ESR1* expression was reported in tamoxifen-resistant samples of a previous study by Kim *et al.* (41), and *ESR1* was found to act as a hub gene in the PPI network. All of these findings suggest that *ESR1* has a central role in resistance to tamoxifen.

The RAN-binding protein 2 (*RANBP2*) is located at chromosomal region 2q13 and was initially considered to be a regulated factor of nucleo-cytoplasmic trafficking (42). *RANBP2* encodes a nucleoporin with 358-kDa that functions in nuclear export or import, including mitotic progression (43,44). Felix *et al.* reported that, compared to plasma cells, the overexpression of *RANBP2* was found in >50% of multiple myeloma cases (45). In addition, the gene is implicated in some tumorigenic pathways, for example, indirectly in the p53 and PI3K/Akt pathways (46,47). Recently, there has been increasing evidence to show that the upregulation of *RANBP2* promotes cancer cell growth in cholangiocarcinoma, cervical cancer, and hepatocellular carcinoma (48-50). From the present study, it is evident that *RANBP2* serving as an upregulated gene may impact tamoxifen resistance. Due to the function of *RANBP2* in cancer, it is likely to become a viable therapeutic approach for treating tamoxifen resistance.

The protein kinase C alpha (*PRKCA*) gene, one of the protein kinase C (PKC) family members, is activated by a variety of stimuli, including tyrosine kinase receptors and guanine nucleotide-binding protein-coupled receptors, and plays critical roles in many different cellular processes, including the cellular functions of proliferation, apoptosis, and differentiation (51). Kim *et al.* (52) indicated that PKC-α mediated cell invasion and migration in breast cancer cells. Moreover, it was found that PKC-α could inhibit ER-α expression by suppressing c-Jun phosphorylation and that the level of PKC-α phosphorylation was significantly increased in the tamoxifen-resistant cell line (41). These data imply that PKC-α is a potential biomarker in tamoxifen resistance.

Conclusions

A total of 438 DEGs were revealed in tamoxifen-resistant breast cancer and tamoxifen-sensitive samples using gene expression profiles. Among these DEGs, *MAPK1*, *ESR1*, *SMARCA4*, *RANBP2*, and *PRKCA* were found to act as hub genes, and they may participate in the important biological processes and pathways involved in the mechanism of tamoxifen resistance. Further research, however, is required to validate these potential therapies.

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Acknowledgments

First and foremost, we extend our appreciation to Professor Jun Liu for her guidance and discussions. Second, we would like to thank Professor Qing Chen, as we would not have been able to continue this study without her patience and encouragement.
Funding: None.

Footnote

Reporting Checklist: The authors have completed the STREGA reporting checklist. Available at https://dx.doi.org/10.21037/tcr-21-1276

Peer Review File: Available at https://dx.doi.org/10.21037/tcr-21-1276

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/tcr-21-1276). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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(English Language Editors: K. Gilbert and J. Gray)

Cite this article as: Wang X, Wang S. Identification of key genes involved in tamoxifen-resistant breast cancer using bioinformatics analysis. Transl Cancer Res 2021;10(12):5246-5257. doi: 10.21037/tcr-21-1276

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