European Review for Medical and Pharmacological Sciences 2024; 28: 603-614

Identification of new subtypes and potential genetic signatures in triple-negative breast cancer using weighted gene co-expression network analysis

J. HUANG, L.-Y. LIAO, W.-J. JIANG, Y.-H. LI, B.-M. LU, Z.-P. WEN, F.-J. LI, D.-L. FANG, G.-M. LU

Department of Gland Surgery, The Affiliated Hospital of Youjiang Medical University for Nationalities, Baise, Guangxi, China

J. Huang, L.Y. Liao, and W.J. Jiang contributed equally to this work, and they are all first authors D.L. Fang and G.M. Lu contribute the same to the article and are the corresponding authors

Abstract. – OBJECTIVE: Triple-negative breast cancer (TNBC) is a heterogeneous disease with aggressive behavior and poor prognosis. Here, we used gene expression profiling to define new subtypes of TNBC, which may improve prevention and treatment through personalized medicine.

MATERIALS AND METHODS: Gene expression profiles from the public datasets GSE76250, GSE61724, GSE61723, and GES76275 were subjected to co-expression analysis to identify differentially expressed genes (DEGs) between TNBC and non-TNBC tissues. Consistency clustering was used to define TNBC subtypes, whose correlation with gene modules was analyzed. Enrichment analysis was used to identify module genes' biological functions and pathways. Single-sample gene set enrichment analysis was used to assess immune cell infiltration in the different TNBC subtypes, and the ChAMP package was used to examine methylation sites in TNBC.

RESULTS: A total of 4,958 DEGs in TNBC were identified, which showed the same expression differences across all datasets as in the dataset GSE76250 and clustered into 9 co-expression modules. TNBC samples clustered into two subtypes based on nine hub genes from the modules. Class I showed the most significant correlation with module 1, whose genes were related mainly to interleukin-1 response, while class II showed the most significant correlation with module 6, whose genes were related mainly to the transforming growth factor-β pathway. Class I was significantly enriched in cell cycle and DNA replication, and tumors of this subtype showed lower immune cell infiltration than class II tumors. Tumor infiltration by Th2 cells correlated positively with the expression of MCM10 and negatively with the expression of PREX2. A greater methylation of CIDEC, DLC1, EDNRB, EGR2 and SRPK1 correlated with better prognosis.

CONCLUSIONS: Class I TNBC, for which a useful biomarker is MCM10, may be associated with a worse prognosis than class II TNBC, for which PREX2 may serve as a biomarker.

Key Words:
Triple-negative breast cancer (TNBC), Bioinformatics analysis, Genetic signatures, WGCNA.

Introduction

Breast cancer is the most common malignant tumor and a common cause of cancer death in women all over the world[1]. In 2018, for example, there were approximately 2.1 million new cases and 627,000 deaths in the world[2]. Recurrence and metastasis are the main causes of death.

Triple-negative breast cancer (TNBC), which accounts for 10-15% of all cases of breast cancer[3], is defined as tumors that do not express the estrogen receptor, progesterone receptor, or human epidermal growth factor receptor-2 (*HER2*). TNBC tumors are more aggressive, show a more advanced tumor grade, and are more likely to metastasize to lymph nodes than other types of breast cancer[4]. TNBC does not respond to chemotherapies targeting the endocrine system or *HER2*, and it is associated with

Corresponding Authors: D.-L. Fang, MD; e-mail: fangdalang@stu.gxmu.edu.cn; G.M. Lu, MD; e-mail: luguanming@yeah.net

a high mortality rate[5]. Even after resection of primary TNBC tumors in the event of timely diagnosis, up to 30% of patients suffer relapse in distant organs[6].

The poor efficacy of existing therapies against TNBC is due in part to its molecular heterogeneity, prompting efforts to define TNBC subtypes that differ in therapy response and prognosis[7,8]. Further molecular understanding of TNBC and its potential subtypes may allow more effective, personalized treatment[9,10]. So far, TNBC subtypes have been explored using gene expression profiling[11]. Studies of other cancers have also shown DNA methylation markers to be useful for personalizing treatment[12,13], since DNA methylation affects gene expression and nuclear structure[14]. Considering methylation markers may help refine the classification of TNBC subtypes.

The present study applied weighted gene co-expression network analysis (WGCNA) and DNA methylation profiling to define TNBC subtypes. WGCNA can identify relationships between gene sets and clinical features in order to identify candidate biomarkers[15]. The results may help guide personalized treatment of the disease and future efforts to develop novel therapies.

Materials and Methods

Data Processing

Gene expression profiles in the datasets GSE76250, GSE61724, GSE61723 and GSE76275 in the Gene Expression Omnibus (GEO) database were downloaded. GSE76250 contains 165 TNBC samples and 33 paired normal breast tissues, GSE61724, 64 primary TNBC samples and 4 normal adjacent tissues, GSE61723, 48 TNBC samples and 17 normal adjacent tissues, and GSE76275, 198 TNBC samples and 67 non-TNBC tumor samples. Data were analyzed using the Robust Multi-array Average method in the Affy package[16] in R version 3.1.1. Genes differentially expressed between TNBC and non-TNBC tissue (DEGs) were identified using the *limma* package[17] in R, with a filtering threshold of $p = 0.05$.

WGCNA

Co-expression of DEGs was analyzed using the *WGCNA* package in R as described[15], using a correlation coefficient threshold of 0.9 and a soft threshold of 6. Hub genes in each module were identified according to the degree value.

Identification of TNBC Subtypes

The ConsensusClusterPlus package[18] in R was used to cluster the expression of hub genes in TNBC samples, leading to the classification of 198 TNBC samples from the GSE76275 dataset into class I or class II.

Enrichment Analysis

Module genes were analyzed for enrichment of Gene Ontology (GO) functions and for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways using the "enrichgo" and "enrichkegg" functions in the clusterProfiler package[19] in R. In both cases, the cut-off criterion for enrichment was $p = 0.05$.

The clusterProfiler package was also used to conduct gene set enrichment analysis (GSEA) between TNBC and non-TNBC tumors. The "subtypeGSEA" function was used to analyze the top pathways that were up- or down-regulated in TNBC relative to non-TNBC.

Immune Infiltration Score

Single-sample GSEA was used to quantify the extent of immune cell infiltration of TNBC samples based on 24 immune cell signatures[20]. The *limma* package was used to determine differences between class I and class II TNBC.

DNA Methylation

The *cAMP* package in R was used to identify differences in methylation between the 105 TNBC and 4 normal samples in the GSE78758 dataset. The overall survival of patients with different methylation profiles was examined using the survival package, and forest plots were drawn using the forest plot package.

Statistical Analysis

All statistical analyses were performed using R software. The difference analysis and correlation analysis were performed *via* Wilcoxon and Spearman correlation, respectively. $p < 0.05$ was considered statistically significant.

Results

A flowchart showing the various analyses in this study is shown in Figure 1.

DEGs in TNBC

We identified DEGs between TNBC and control samples in the GSE76250, GES61724,

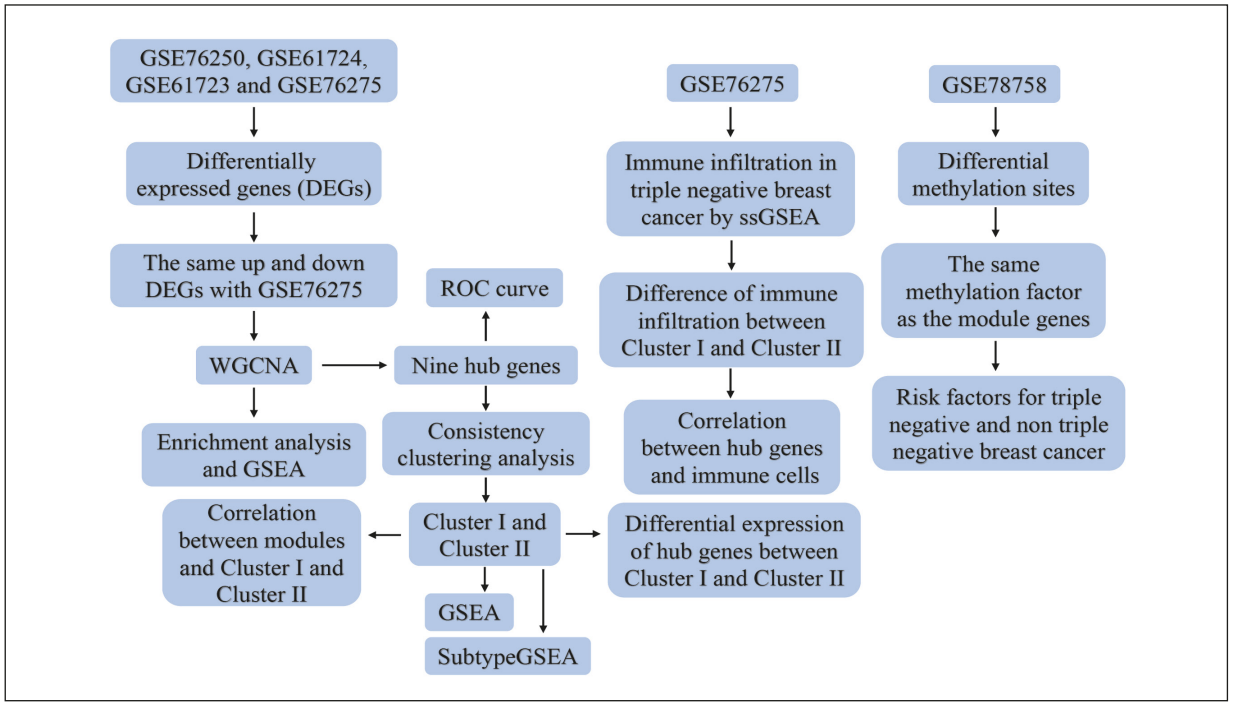

Figure 1. Flowchart of analyses. DEG, differentially expressed genes; GSEA, gene set enrichment analysis; ROC, receiver operating characteristic; ssGSEA, single-sample GSEA; WGCNA, weighted gene co-expression network analysis.

GSE61723 and GE76275 datasets (Figure 2A). The GSE76250 dataset showed the same 3,176 up-regulated genes and 2,359 down-regulated genes as analysis of all datasets combined (Figure 2B). Applying a correlation coefficient threshold of 0.9 and a soft threshold of 6 (Figure 2C), 4,958 DEGs clustered into 9 co-expression modules were identified (Figure 2D). Hub genes in these modules were obtained (Table I).

TNBC Subtypes

All hub genes showed an area under the receiver operating characteristic curve >0.6 for distinguishing TNBC from non-TNBC tissue (Figure 3A), and the TNBC samples were divided into classes I and II based on nine hub genes (Figure 3B). Module 1 (MEturquoise) showed the strongest positive correlation with class I and the weakest correlation with class II. Conversely, module 6 (MEbrown) showed the strongest positive correlation with class II and the weakest correlation with class I (Figure 3C). *MCM10* differentiated TNBC from non-TNBC better than *PREX2* (Figure 3D). Hub gene *MCM10* in module 1 was up-regulated in TNBC, while hub gene *PREX2* in module 6 was down-regulated in TNBC (Figure 3E). *MCM10* and *PREX2* were expressed at higher levels in class I than class II TNBC (Figure 3F).

Biological Functions Related to Class I or II TNBC

Enrichment analysis showed that module genes were enriched mainly in cellular response to oxygen levels, response to interleukin-1, positive regulation of MAPK cascade and other biological processes (Figure 4A). KEGG pathways included mainly p53 signaling, carbon metabolism, and MAPK signaling (Figure 4B). GSEA identified the same four KEGG pathways differing between TNBC and non-TNBC as between classes I and II of TNBC (Figure 4C-D). Many module genes were implicated in these pathways (Figure 4E). In addition, pathways involving the cell cycle and DNA replication were up-regulated in class I TNBC relative to class II TNBC or in TNBC relative to non-TNBC (Figure 4F).

Immune Cell Infiltration in Class I or II TNBC

Single-sample GSEA showed that TNBC tumors contained lower numbers of most immune cell types than non-TNBC tumors (Figure 5A).

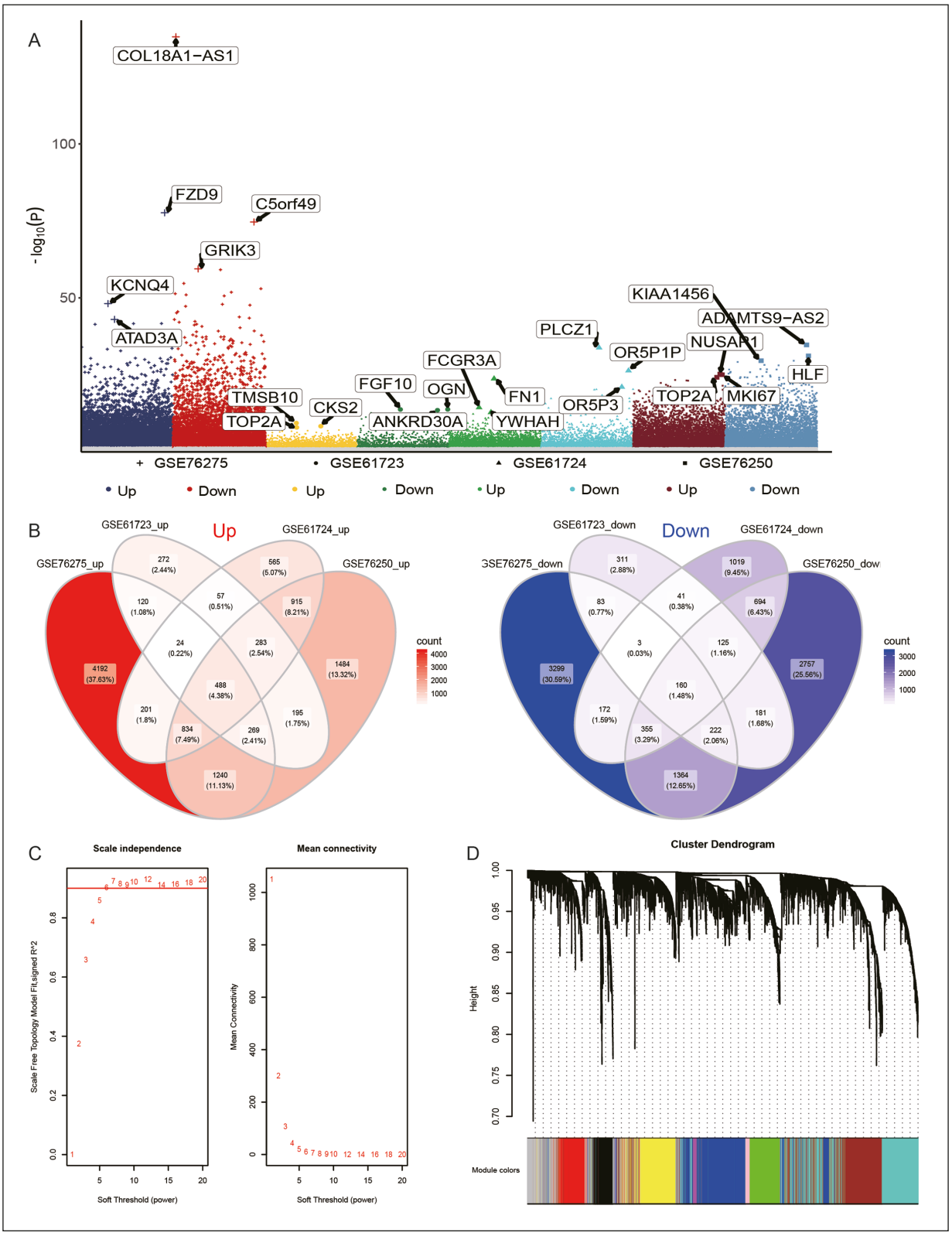

Figure 2. Differentially expressed genes and their co-expression network in triple-negative breast cancer. **A**, Genes differentially expressed between triple-negative breast cancer and non-triple-negative breast cancer in GSE76250, GSE61724, GSE61723 and GSE76275 datasets. **B**, Veen map of four groups of differentially expressed genes. **C**, Soft thresholding of the co-expression network. **D**, Cluster tree of genes in the co-expression modules.

Table I. Hub genes of co-expression modules.

Colour	HubGenes	Module
Black	*SNX20*	m3
Blue	*BAG6*	m4
Brown	*PREX2*	m6
Green	*BCRP3*	m5
Magenta	*GRN*	m7
Pink	*ADAM5*	m8
Red	*ARPP21*	m9
Turquoise	*MCM10*	m1
Yellow	*ACADSB*	m2

Class I TNBC tumors contained fewer infiltrating cells than class II tumors. K-means clustering defined a potential network of interactions among tumor-infiltrating immune cells (Figure 5B). Abundances of most immune cell types correlated negatively with the hub gene *MCM10* and positively with the hub gene *PREX2* (Figure 5C). Conversely, the numbers of Th2 cells correlated positively with *MCM10* and negatively with *PREX2* in classes I and II (Figure 5D). In class I TNBC, the strongest correlation between immune cell types was between cytotoxic cells and T cells; in class II, the strongest correlation was between macrophages and neutrophils (Figure 5E).

Methylation Patterns in TNBC

Sites of DNA methylation differing between TNBC and control tissues clustered into four groups (Figure 6A-B). *DMPS* was hypomethylated in 94.26% of TNBC tissues relative to control tissues. There were 10% of methylation sites located on chromosome 1 (Figure 6C). Among all differentially methylated genes, 102 were module genes defined as potential methylation markers (Figure 6D). The following methylation markers significantly influenced overall survival of TNBC patients: *CIDEC* (cg07222243), *DLC1* (cg02126477), *EDNRB* (cg12120741), *EGR2* (cg22867608), and *SRPK1* (cg12215478) (Figure 6E). Hypermethylated *CIDEC* was associated with longer overall survival, while hypermethylated *DLC1*, hypermethylated *EDNRB*, hypermethylated *EGR2* and hypomethylated *SRPK1* were associated with shorter overall survival.

Discussion

Our RNAseq-based comparison of publicly available data from TNBC and non-TNBC identified many DEGs that may be associated with TNBC. Using WGCNA, we clustered these DEGs into 9 co-expression modules, each of which may represent a disease pathway[21]. The hub genes from each module showed a good ability to differentiate TNBC from non-TNBC, and their cluster analysis led us to identify two classes of TNBC.

Module 1 showed the strongest correlation with class I TNBC, while module 6 showed the strongest correlation with class II. The hub gene *MCM10* in module 1 was up-regulated in TNBC relative to non-TNBC, and it was up-regulated in class I TNBC relative to class II. Previous work has shown *MCM10* to be up-regulated in tumor tissues[22], and its expression promotes the development of breast cancer and correlates positively with poor prognosis[23,24]. The hub gene *PREX2* in module 6 was down-regulated in TNBC relative to control and down-regulated in class II TNBC relative to class I TNBC. Consistent with our results, *PREX2* may promote cell migration and invasion, contributing to breast cancer[25,26]; indeed, its overexpression has been associated with poor prognosis of breast cancer patients[27]. Our results suggest that patients with class I TNBC may suffer a worse prognosis than those with class II disease.

We used enrichment analysis to identify molecular pathways that may contribute to TNBC, and our results are consistent with the literature. Cellular response to oxygen levels has been implicated in the development of breast cancer[28,29], and interleukin-1 may help predict the risk of bone metastasis in breast cancer patients[30,31]. Signaling mediated by p53 may help regulate progression of early breast cancer, especially TNBC[32,33]. Changes in carbon metabolism can lead to abnormal DNA methylation and damage DNA integrity, promoting the occurrence of cancer[34]. Consistent with our analysis of enrichment in module 6, transforming growth factor-β has been shown to inhibit proliferation and induce apoptosis in early breast cancer, as well as promote tumor invasiveness in later stages of the disease[35]. Activation of MAPK signaling promotes the proliferation and metastasis of breast cancer cells[36], while activation of Wnt signaling contributes to tumor occurrence and recurrence[37,38]. Our GSEA showed the cell cycle and DNA replication, which have been implicated in breast cancer[39-41], to be up-regulated in class I TNBC relative to class II.

The immune microenvironment within TNBC tissues is highly heterogeneous[42], and understanding this heterogeneity may be important

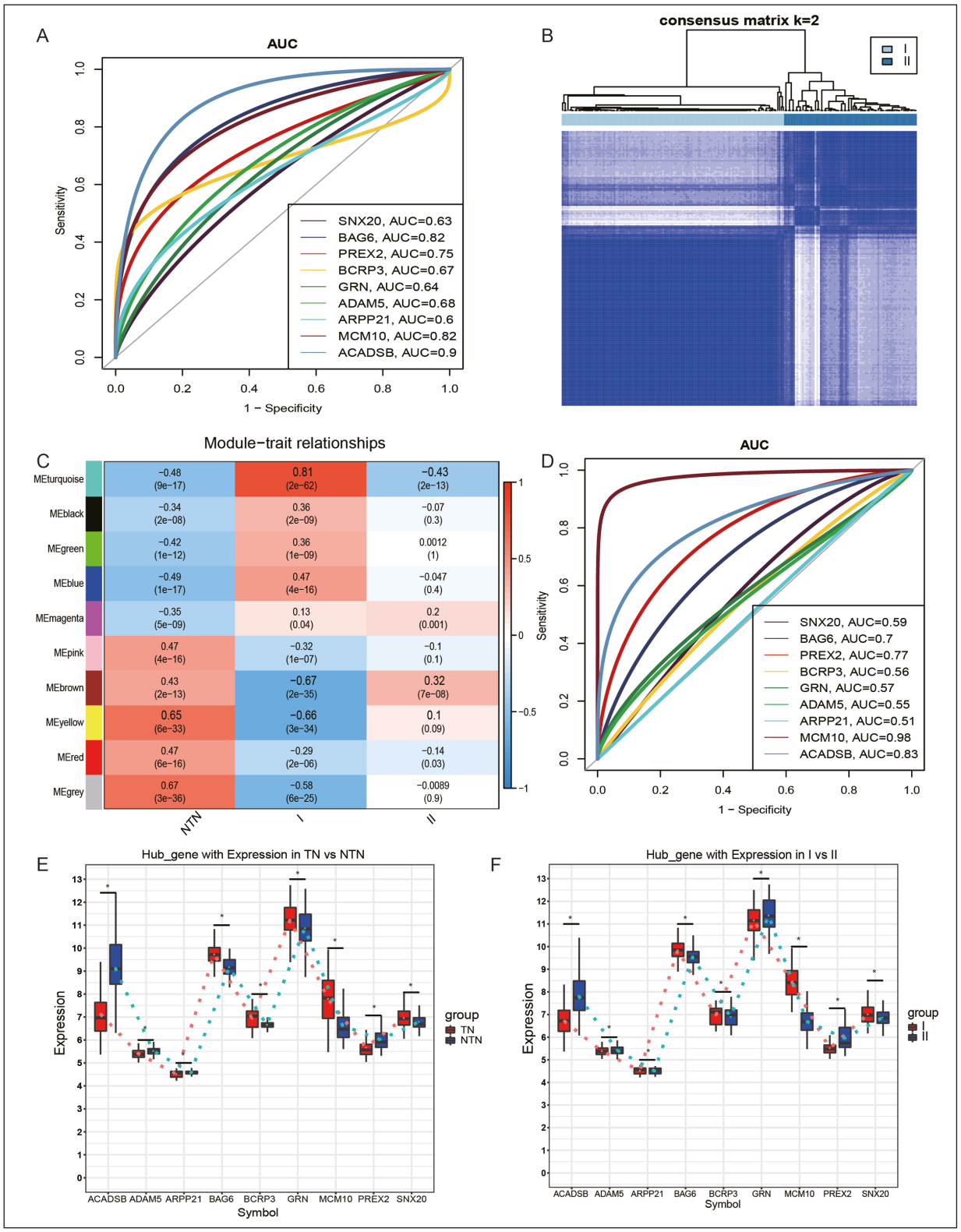

Figure 3. Identification of new subtypes of triple-negative breast cancer. **A**, Receiver operating characteristic curves of hub genes in triple-negative breast cancer and non-triple-negative breast cancer. AUC, area under the curve. **B**, Consistency clustering analysis was conducted based on 9 hub genes. **C**, Correlation between co-expression modules and phenotype. **D**, Receiver operating characteristic curves of hub genes in classes I and II. **E**, Expression of hub genes in triple-negative breast cancer and non-triple-negative breast cancer. **F**, Expression of hub genes in classes I and II triple-negative breast cancer. *$p < 0.05$.

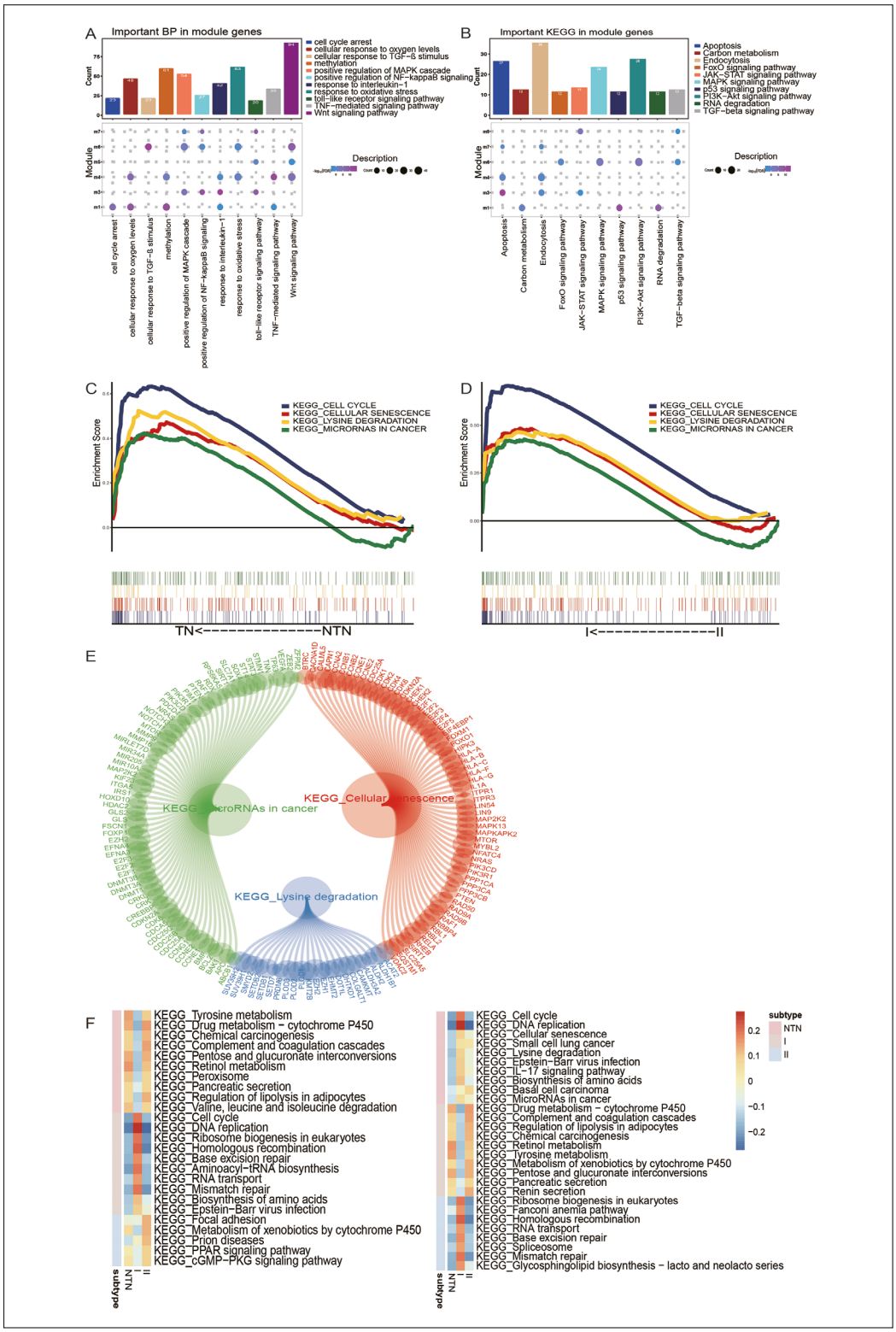

Figure 4. Biological functions and signaling pathways of module genes. **A**, Important Gene Ontology biological processes enriched in the module genes. **B**, Important Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched in the module genes. **C**, Gene set enrichment analysis of KEGG pathways in triple-negative breast cancer and non-triple-negative breast cancer. **D**, Gene set enrichment analysis of KEGG pathways in class I and class II triple-negative breast cancer. **E**, Module genes involved in the same four KEGG pathways between GSEA and KEGG enrichment. **F**, The signaling pathways in non-triple-negative breast cancer and class I and II triple-negative breast cancer were up-regulated or down-regulated at the same time.

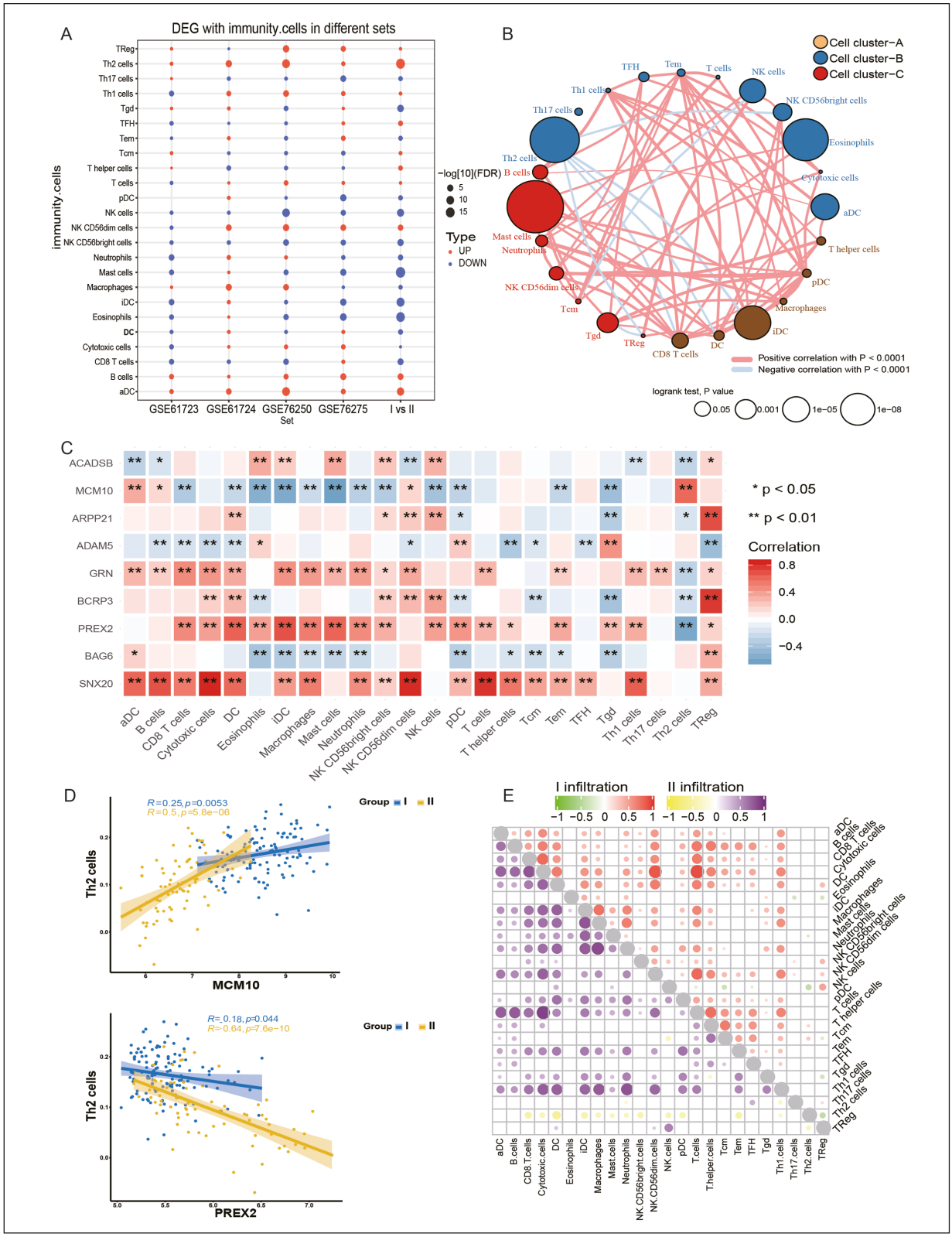

Figure 5. Immune cell infiltration of triple-negative breast cancer tumors. **A**, Differences in immune cell infiltration of tumors between triple-negative breast cancer and non-triple-negative breast cancer, and between class I and class II triple-negative breast cancer. **B**, Correlations between numbers of different immune cell types in tumors. **C**, Correlations between module hub gene expression and numbers of different immune cell types in tumors. **D**, Correlations of numbers of infiltrating Th2 cells with expression of the hub genes MCM10 and PREX2. **E**, Correlations of numbers of different immune cell types infiltrating class I or class II tumors.

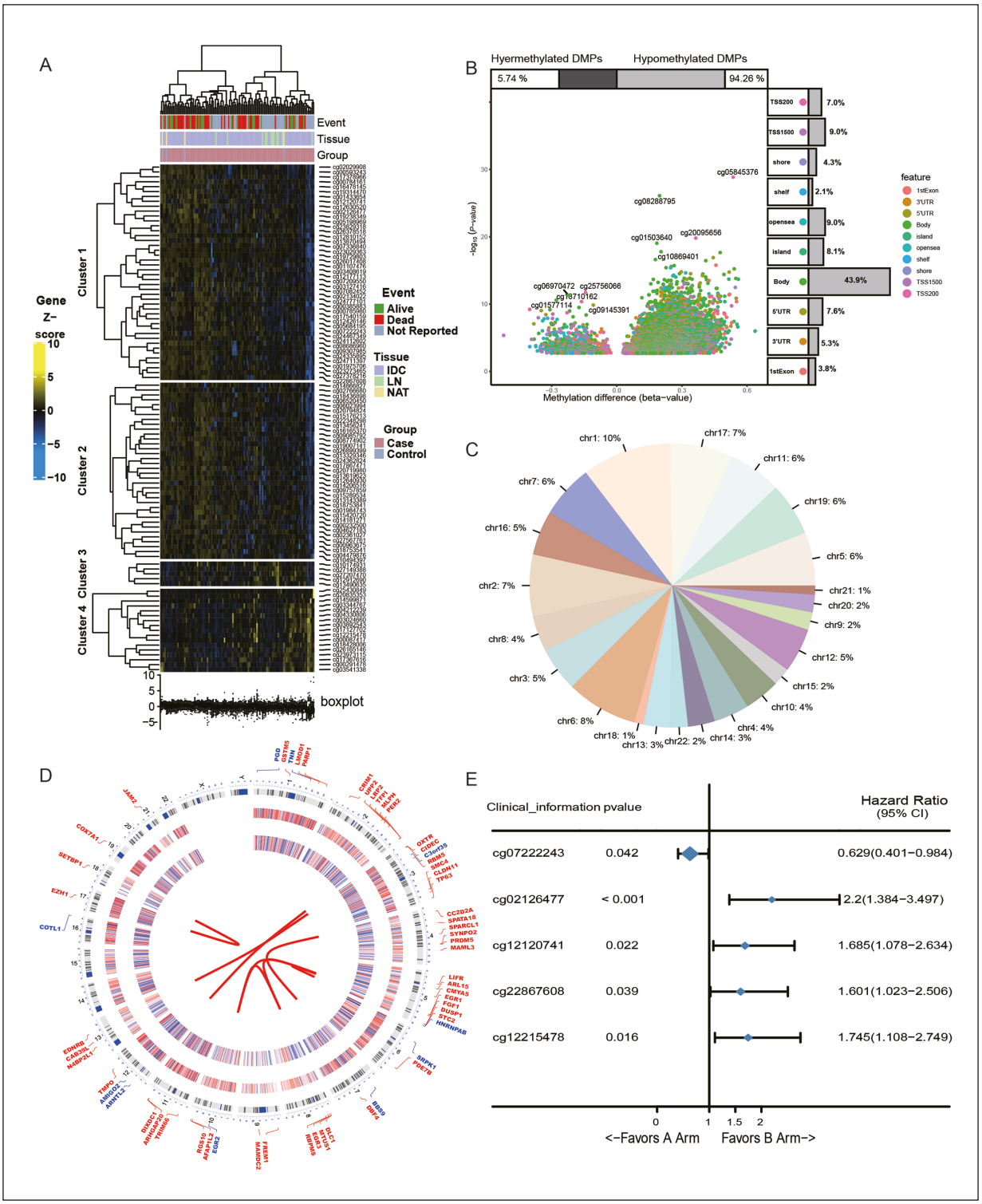

Figure 6. Altered methylation in triple-negative breast cancer. **A**, Sites of methylation in triple-negative breast cancer and non-triple-negative breast cancer. **B**, Differences in methylation sites between triple-negative breast cancer and controls. **C**, Distribution of methylation sites across chromosomes (chr). **D**, Circular plot of methylation markers, generated using OmicCircos. Genes in red are hypermethylated in TNBC relative to non-TNBC; genes in blue, hypomethylated. **E**, Forest plot of methylation markers to identify ones associated with overall survival.

for improving treatment. For example, higher numbers of lymphocytes in the tumor stroma have been associated with higher survival rates[43]. We found evidence that class I TNBC tumors show relatively low infiltration by immune cells. Expression of the hub gene *MCM10* correlated negatively with the abundance of most types of tumor-infiltrating immune cells, but positively with Th2 cells. Conversely, expression of the hub gene *PREX2* correlated negatively with Th2 cells. Shifting the balance between Th1 and Th2 populations towards Th2 may promote the growth of breast cancer cells[44,45]. Such differences in immune cell infiltration may help explain the observed difference in overall survival between class I and II TNBC.

Many cancers, including TNBC, feature hypermethylation of CpG islands in the promoter regions of genes encoding key growth regulators[46]. Therefore, we examined methylation patterns in TNBC in order to identify potential markers to guide diagnosis and treatment. Consistent with our results, *CIDEC* has been shown to be down-regulated in breast cancer[47], while *EDNRB* is up-regulated in TNBC cells[48]. Hypermethylation of the *DLC1* promoter may help drive breast cancer[49]. *EGR2* may be an oncogene of invasive breast cancer[50], and high *SRPK1* expression has been associated with increased breast cancer invasiveness and poor prognosis[51]. Further work is needed to clarify how methylation contributes to TNBC and its different subtypes.

Future work should expand on our analyses since this study was unable to compare methylation profiles between TNBC and non-TNBC[52,53], nor did we verify our genetic findings with cellular or biochemical analyses. Clinical studies should also compare the prognosis of patients with class I or II TNBC.

Conclusions

We screened 9 co-expression modules of TNBC-related genes and used the module hub genes to classify TNBC into classes I and II. Class I showed the strongest correlation with module 1, while class II showed the strongest correlation with module 6. Class I TNBC tumors appear to show less infiltration by immune cells than class II tumors. The intra-tumor abundance of most types of immune cells correlated negatively with expression of the hub gene *MCM10* from module 1 but positively with expression of the hub gene *PREX2* from module 6. Our results suggest that class I TNBC may be associated with a worse prognosis than class II disease, which should be confirmed in clinical studies. However, based on the results of our study, the biomarkers of different subtypes of TNBC may have important guiding significance for the diagnosis and treatment of TNBC in the future. Our methylation analyses suggest that the difference in overall survival may relate to differences in methylation of *CIDEC, DLC1, EDNRB, EGR2,* and *SRPK1.*

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. All the authors were informed and agreed to submit the manuscript for publication.

Authors' Contribution

I) Conception and design: J. Huang, L. Liao and W. Jiang; (II) Administrative support: G. Lu and D. Fang; (III) Provision of study materials or patients: Y. Li, B. Lu, and L. Liao; (IV) Collection and assembly of data: J. Huang; (V) Data analysis and interpretation: G. Lu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval

All data in this paper are from public databases that have been published and do not involve new human and animal experiments, so no ethical proof is required. 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.

Funding

This study was supported by: A project funded by Project of National Natural Science Foundation of China (No. 32060208). General program of Guangxi Natural Science Foundation (No. 2019JJA140071). The First Batch of High-level Talent Scientific Research Projects of the Affiliated Hospital of Youjiang Medical University for Nationalities in 2019 (Contract No. R20196307). Project of Youjiang Medical University for nationality (No. YY2019ky001).

Informed Consent

Not applicable.

References

1) Lee KL, Kuo YC, Ho YS, Huang YH. Triple-Negative Breast Cancer: Current Understanding and Future Therapeutic Breakthrough Targeting Cancer Stemness. Cancers (Basel) 2019; 11: 1334.
2) Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.
3) Shi Y, Jin J, Ji W, Guan X. Therapeutic landscape in mutational triple negative breast cancer. Mol Cancer 2018; 17: 99.
4) Cao L, Niu Y. Triple negative breast cancer: special histological types and emerging therapeutic methods. Cancer Biol Med 2020; 17: 293-306.
5) Qin JJ, Yan L, Zhang J, Zhang WD. STAT3 as a potential therapeutic target in triple negative breast cancer: a systematic review. J Exp Clin Cancer Res 2019; 38: 195.
6) McAllister SS, Gifford AM, Greiner AL, Kelleher SP, Saelzler MP, Ince TA, Reinhardt F, Harris LN, Hylander BL, Repasky EA, Weinberg RA. Systemic endocrine instigation of indolent tumor growth requires osteopontin. Cell 2008; 133: 994-1005.
7) Lehmann BD, Jovanovic B, Chen X, Estrada MV, Johnson KN, Shyr Y, Moses HL, Sanders ME, Pietenpol JA. Refinement of Triple-Negative Breast Cancer Molecular Subtypes: Implications for Neoadjuvant Chemotherapy Selection. PLoS One 2016; 11: e0157368.
8) Burstein MD, Tsimelzon A, Poage GM, Covington KR, Contreras A, Fuqua SA, Savage MI, Osborne CK, Hilsenbeck SG, Chang JC, Mills GB, Lau CC, Brown PH. Comprehensive genomic analysis identifies novel subtypes and targets of triple-negative breast cancer. Clin Cancer Res 2015; 21: 1688-1698.
9) Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, Pietenpol JA. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 2011; 121: 2750-2767.
10) Lehmann BD, Pietenpol JA. Identification and use of biomarkers in treatment strategies for triple-negative breast cancer subtypes. J Pathol 2014; 232: 142-150.
11) Yam C, Mani SA, Moulder SL. Targeting the Molecular Subtypes of Triple Negative Breast Cancer: Understanding the Diversity to Progress the Field. Oncologist 2017; 22: 1086-1093.
12) Basse C, Arock M. The increasing roles of epigenetics in breast cancer: Implications for pathogenicity, biomarkers, prevention and treatment. Int J Cancer 2015; 137: 2785-2794.
13) Yang X, Lay F, Han H, Jones PA. Targeting DNA methylation for epigenetic therapy. Trends Pharmacol Sci 2010; 31: 536-546.
14) Absmaier M, Napieralski R, Schuster T, Aubele M, Walch A, Magdolen V, Dorn J, Gross E, Harbeck N, Noske A, Kiechle M, Schmitt M. PITX2 DNA-methylation predicts response to anthracycline-based adjuvant chemotherapy in triple-negative breast cancer patients. Int J Oncol 2018; 52: 755-767.
15) Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 2008; 9: 559.
16) Gautier L, Cope L, Bolstad BM, Irizarry RA. affy--analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 2004; 20: 307-315.
17) Yu J, Wu X, Huang K, Zhu M, Zhang X, Zhang Y, Chen S, Xu X, Zhang Q. Bioinformatics identification of lncRNA biomarkers associated with the progression of esophageal squamous cell carcinoma. Mol Med Rep 2019; 19: 5309-5320.
18) Wilkerson MD, Hayes DN. ConsensusClusterPlus: a class discovery tool with confidence assessments and item tracking. Bioinformatics 2010; 26: 1572-1573.
19) Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS 2012; 16: 284-287.
20) Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, Angell H, Fredriksen T, Lafontaine L, Berger A, Bruneval P, Fridman WH, Becker C, Pages F, Speicher MR, Trajanoski Z, Galon J. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity 2013; 39: 782-795.
21) Yao Q, Song Z, Wang B, Qin Q, Zhang JA. Identifying Key Genes and Functionally Enriched Pathways in Sjogren's Syndrome by Weighted Gene Co-Expression Network Analysis. Front Genet 2019; 10: 1142.
22) Tang J, Kong D, Cui Q, Wang K, Zhang D, Gong Y, Wu G. Prognostic Genes of Breast Cancer Identified by Gene Co-expression Network Analysis. Front Oncol 2018; 8: 374.
23) Yang WD, Wang L. MCM10 facilitates the invaded/migrated potentials of breast cancer cells via Wnt/beta-catenin signaling and is positively interlinked with poor prognosis in breast carcinoma. J Biochem Mol Toxicol 2019; 33: e22330.
24) Mahadevappa R, Neves H, Yuen SM, Jameel M, Bai Y, Yuen HF, Zhang SD, Zhu Y, Lin Y, Kwok HF. DNA Replication Licensing Protein MCM10 Promotes Tumor Progression and Is a Novel Prognostic Biomarker and Potential Therapeutic Target in Breast Cancer. Cancers (Basel) 2018; 10: 282.
25) Mense SM, Barrows D, Hodakoski C, Steinbach N, Schoenfeld D, Su W, Hopkins BD, Su T, Fine B, Hibshoosh H, Parsons R. PTEN inhibits PREX2-catalyzed activation of RAC1 to restrain tumor cell invasion. Sci Signal 2015; 8: ra32.
26) Srijakotre N, Man J, Ooms LM, Lucato CM, Ellisdon AM, Mitchell CA. P-Rex1 and P-Rex2 RacGEFs and cancer. Biochem Soc Trans 2017; 45: 963-977.
27) Pandiella A, Montero JC. Molecular pathways: P-Rex in cancer. Clin Cancer Res 2013; 19: 4564-4569.

28) Gurer-Orhan H, Ince E, Konyar D, Saso L, Suzen S. The Role of Oxidative Stress Modulators in Breast Cancer. Curr Med Chem 2018; 25: 4084-4101.

29) Johar R, Sharma R, Kaur A, Mukherjee TK. Role of Reactive Oxygen Species in Estrogen Dependant Breast Cancer Complication. Anticancer Agents Med Chem 2015; 16: 190-199.

30) Holen I, Lefley DV, Francis SE, Rennicks S, Bradbury S, Coleman RE, Ottewell P. IL-1 drives breast cancer growth and bone metastasis in vivo. Oncotarget 2016; 7: 75571-75584.

31) Tulotta C, Ottewell P. The role of IL-1B in breast cancer bone metastasis. Endocr Relat Cancer 2018; 25: R421-R434.

32) Chang JW, Kuo WH, Lin CM, Chen WL, Chan SH, Chiu MF, Chang IS, Jiang SS, Tsai FY, Chen CH, Huang PH, Chang KJ, Lin KT, Lin SC, Wang MY, Uen YH, Tu CW, Hou MF, Tsai SF, Shen CY, Tung SL, Wang LH. Wild-type p53 upregulates an early onset breast cancer-associated gene GAS7 to suppress metastasis via GAS7-CYFIP1-mediated signaling pathway. Oncogene 2018; 37: 4137-4150.

33) Duffy MJ, Synnott NC, Crown J. Mutant p53 in breast cancer: potential as a therapeutic target and biomarker. Breast Cancer Res Treat 2018; 170: 213-219.

34) Gong Z, Yao S, Zirpoli G, David Cheng TY, Roberts M, Khoury T, Ciupak G, Davis W, Pawlish K, Jandorf L, Bovbjerg DH, Bandera EV, Ambrosone CB. Genetic variants in one-carbon metabolism genes and breast cancer risk in European American and African American women. Int J Cancer 2015; 137: 666-677.

35) Zhao Y, Ma J, Fan Y, Wang Z, Tian R, Ji W, Zhang F, Niu R. TGF-beta transactivates EGFR and facilitates breast cancer migration and invasion through canonical Smad3 and ERK/Sp1 signaling pathways. Mol Oncol 2018; 12: 305-321.

36) Yang XL, Liu KY, Lin FJ, Shi HM, Ou ZL. CCL28 promotes breast cancer growth and metastasis through MAPK-mediated cellular anti-apoptosis and pro-metastasis. Oncol Rep 2017; 38: 1393-1401.

37) Huang Y, Yang L, Pei YY, Wang J, Wu H, Yuan J, Wang L. Overexpressed ACBD3 has prognostic value in human breast cancer and promotes the self-renewal potential of breast cancer cells by activating the Wnt/beta-catenin signaling pathway. Exp Cell Res 2018; 363: 39-47.

38) Krishnamurthy N, Kurzrock R. Targeting the Wnt/beta-catenin pathway in cancer: Update on effectors and inhibitors. Cancer Treat Rev 2018; 62: 50-60.

39) Mitsis M, Alexiou GA, Vartholomatos E, Markopoulos G, Lazari D, Hodaj E, Nastos D, Zagorianakou P, Galani V, Kyritsis AP. N-(p-coumaroyl) serotonin induces cell cycle arrest and apoptosis in breast cancer cells. J Buon 2018; 23: 129-133.

40) Liu B, Liu Y, Wang Y, Xie C, Gan M, Han T, Cao J, Wang J. CyclinB1 deubiquitination by USP14 regulates cell cycle progression in breast cancer. Pathol Res Pract 2019; 215: 152592.

41) Lo PK, Lee JS, Liang X, Sukumar S. The dual role of FOXF2 in regulation of DNA replication and the epithelial-mesenchymal transition in breast cancer progression. Cell Signal 2016; 28: 1502-1519.

42) Saraiva DP, Guadalupe Cabral M, Jacinto A, Braga S. How many diseases is triple negative breast cancer: the protagonism of the immune microenvironment. ESMO Open 2017; 2: e000208.

43) Adams TA, Vail PJ, Ruiz A, Mollaee M, McCue PA, Knudsen ES, Witkiewicz AK. Composite analysis of immunological and metabolic markers defines novel subtypes of triple negative breast cancer. Mod Pathol 2018; 31: 288-298.

44) Zhang W, Pan Y, Gou P, Zhou C, Ma L, Liu Q, Du Y, Yang J, Wang Q. Effect of xanthohumol on Th1/Th2 balance in a breast cancer mouse model. Oncol Rep 2018; 39: 280-288.

45) Zhao X, Liu J, Ge S, Chen C, Li S, Wu X, Feng X, Wang Y, Cai D. Saikosaponin A Inhibits Breast Cancer by Regulating Th1/Th2 Balance. Front Pharmacol 2019; 10: 624.

46) Yu J, Qin B, Moyer AM, Nowsheen S, Liu T, Qin S, Zhuang Y, Liu D, Lu SW, Kalari KR, Visscher DW, Copland JA, McLaughlin SA, Moreno-Aspitia A, Northfelt DW, Gray RJ, Lou Z, Suman VJ, Weinshilboum R, Boughey JC, Goetz MP, Wang L. DNA methyltransferase expression in triple-negative breast cancer predicts sensitivity to decitabine. J Clin Invest 2018; 128: 2376-2388.

47) Malvia S, Bagadi SAR, Pradhan D, Chintamani C, Bhatnagar A, Arora D, Sarin R, Saxena S. Study of Gene Expression Profiles of Breast Cancers in Indian Women. Sci Rep 2019; 9: 10018.

48) Gu X, Han S, Cui M, Xue J, Ai L, Sun L, Zhu X, Wang Y, Liu C. Knockdown of endothelin receptor B inhibits the progression of triple-negative breast cancer. Ann N Y Acad Sci 2019; 1448: 5-18.

49) Liu Y, Zhou J, Hu Y, Wang J, Yuan C. Curcumin inhibits growth of human breast cancer cells through demethylation of DLC1 promoter. Mol Cell Biochem 2017; 425: 47-58.

50) Kennedy BM, Harris RE. Cyclooxygenase and lipoxygenase gene expression in the inflammogenesis of breast cancer. Inflammopharmacology 2018. doi: 10.1007/s10787-018-0489-6. Epub ahead of print.

51) van Roosmalen W, Le Devedec SE, Golani O, Smid M, Pulyakhina I, Timmermans AM, Look MP, Zi D, Pont C, de Graauw M, Naffar-Abu-Amara S, Kirsanova C, Rustici G, Hoen PA, Martens JW, Foekens JA, Geiger B, van de Water B. Tumor cell migration screen identifies SRPK1 as breast cancer metastasis determinant. J Clin Invest 2015; 125: 1648-1664.

52) Dimitrov G, Atanasova M, Popova Y, Vasileva K, Milusheva Y, Troianova P. Molecular and genetic subtyping of breast cancer: the era of precision oncology. WCRJ 2022; 9: e2367.

53) Liu HY, Chen Y, Yu YP, Yu Y. Development in biomarkers of breast cancer: a bibliometric analysis from 2011 to 2020. Eur Rev Med Pharmacol Sci 2023 Sep; 27: 8697-8715.