Identification of a Novel Lipid Metabolic-Related Gene Signature with the Tumor Immune Microenvironment for Breast Cancer

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Research

Keywords: Breast cancer, Lipid metabolism, tumor immune 18 microenvironment, immune-related analysis, immunotherapy

Posted Date: October 21st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-977759/v1

Identification of a novel lipid metabolic-related gene signature with the tumor immune microenvironment for breast cancer

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Abstract

Background: Systemic factors can strongly affect how tumor cells behave, grow, and communicate with other cells as increasingly in breast cancer. Lipid metabolic reprogramming is one systemic way tumor cells undergo, however, the formation and dynamics of lipid associated with tumor immune microenvironment (TIME) still remain elusive. The sophisticated bidirectional crosstalk of tumor cells with cancer metabolism, gene expression, and TIME could have the potential to identify novel biomarkers for diagnosing, prognostic, and immunotherapy. This study aimed to construct a prognostic signature to detect the bi-crosstalk between lipid metabolic system and the TIME of breast cancer.

Methods: R software was selected to detect the expression of LRGs and perform the GO/KEGG analysis. Considering the clinical information and pathological features, a predictive nomogram was constructed to predict the survival probability and LASSO Cox regression analysis was performed to construct a prognostic gene signature. The TMB, MSI as well as immune infiltration analysis were performed, in addition, consensus cluster analysis of LRGs were also performed.

Results: These 16 lipid metabolic-related genes (LRGs) were mainly involved in the process of lipid metabolism and fatty acid binding in breast cancer by functional enrichment analysis. Prognosis analysis

identified the prognostic value of FABP7 and NDUFAB1 in breast cancer patients. The prognostic gene signature constructed with FABP7 and NDUFAB1 was significantly related to immune infiltration and could predict the overall survival (OS) rate with above average correctness of breast cancer patients. The analysis of immune infiltration, tumor mutation burden (TMB), and microsatellite instability (MSI) were significantly correlated with FABP7 and NDUFAB1. Consensus clusters analysis identified the up mRNAs were mostly related to the oncogenesis process while the down were associated with immune-related signaling pathways.

Conclusion: We performed a comprehensive analysis to evaluate the lipid metabolic system and identified a signature constructed by two prognostic genes for immunotherapies in breast cancer. Our results also revealed evidence of the vulnerabilities in the bidirectional interplay between the lipid metabolic system and the TIME which may contribute to deciphering the heterogeneity of the TIME in breast cancer.

Keywords: Breast cancer, Lipid metabolism, tumor immune microenvironment, immune-related analysis, immunotherapy.

Introduction

Breast cancer has become the most prevalent malignancy around the world according to the latest statistic results released by the World Health Organization's International Agency for Research on Cancer[1]. This highly heterogeneous malignancy which comprises different subtypes is still seriously threatening the health of women, whereas the triple-negative breast cancer (TNBC) subtype has always been known with the worst prognosis[2-4]. Multiple types of research with numerous efforts have proven that how tumor cells grow, behave and communicate with other cells are not only determined by the characteristics of cancer cells but also by their sophisticated surrounding environment[5,6]. The tumor microenvironment (TME) has been proven to be a dynamic community containing the tumor cells and tumor-related cells[7], TIME (the tumor immune microenvironment) which represents the immune part of TME, are playing the crucial roles and studies have illustrated the complicated bidirectional crosstalk between the tumors cells and the TIME in breast cancer[8].

Reprogramming of energy metabolism that can actively contribute to cancer development has been recognized as one of the cancer hallmarks[9,10]. Carcinogenic events could trigger the regulation of metabolic pathways, which in turn enable the proliferation and survival of cancer cells in the severe microenvironment by providing selective

advantages[11,12]. Lipid metabolism including fatty acid metabolisms, fatty acid transport, and fat differentiation-related signatures are also highly activated in breast cancer cells[13,14], which can both promote and inhibit the oncogenesis and progression of cancer cells by reassigning the nutrient in the microenvironment of breast cancer[15,16].

Immunotherapies such as immune checkpoint blockades and other immunotherapeutic strategies have furnished new hopes for breast cancer patients[17,18], however, the low response rate limits the application of tumor immunotherapy[19]. Hence improved deciphering of how the lipid metabolic system with the TIME modulates cancer development and evasion from the tumor-suppress surveillance may reveal clues for novel anticancer of immunotherapeutic strategies directed to lipid metabolic targets.

Therefore, in this study, we selected 16 lipid metabolic-related genes (LRGs) to detect the bidirectional interplay of the lipid metabolic system of tumor cells with the TIME and construct a prognostic signature to explore the dynamic lipid metabolic signature difference in breast cancer. Our data could provide new evidence for identifying novel prognostic biomarkers of immunotherapies for breast cancer and contributing to reveal the heterogeneity of the TIME in breast cancer.

MATERIALS AND METHODS

Identifying the different expressions of LRGs

16 LRGs in total were selected participating in the lipid metabolic system in breast cancer. The difference in LRGs expression in breast cancer and normal tissues was detected by the limma and reshape2 packages in R software (version 4.0.3)[20,21]. A protein-protein interaction (PPI) network was constructed of these 16 LRGs using the STRING database by the set with a minimum interaction score of 0.9[22].

Functional enrichment analysis

To further identify the function of these LRGs in breast cancer, GO and KEGG databases were selected and the data were performed by functional enrichment analysis. GO (Gene Ontology) database is an online tool for detecting the function of genes for MF (molecular function), BP (biological pathways), and CC (cellular components)[23]. KEGG (Kyoto Encyclopedia of Genes and Genomes) is an open database for detecting the gene pathway enrichments which were performed by Gene Set Enrichment Analysis[24]. To better understand the carcinogenesis of these LRGs, the ClusterProfiler package (version 3.14.0) in R software (version 4.0.3) was performed to analyze the GO function and the KEGG pathway of these potential targets[25].

Construction of the lipid metabolic-related gene prognostic model

The prognostic value of these 16 LRGs was performed by the Kaplan-Meier method and analyzed by Log-Rank test whereas *p*-values and hazard ratios (HRs) with 95% confidence intervals (CIs) were generated. The LRGs which were proven to have a significant prognostic value were selected. Based on the prognostic value of these LRGs, we constructed a prognostic model containing the prognostic LRGs by LASSO Cox regression analysis for breast cancer patients. According to the risk score, the patients with breast cancer were separated into two subgroups with the low-risk and high-risk, and the overall survival (OS) possibility between these two groups was compared by Kaplan–Meier method. The receiver-operating characteristic (ROC) analysis was selected to predict the diagnostic accuracy of each gene. Taking considering the pathological characteristics, a predicted nomogram was developed to predict the 1-year, 3-year, and 5-year overall survival possibility through the forestplot package in R software[26].

The analysis of immune-cell infiltration, immune-checkpoints correlation, tumor mutation burden (TMB), and microsatellite-instability (MSI).

The correlation between the prognostic LRGs and immune-cell infiltration was evaluated using the ssGESA package in R software, for comprehensive analysis of tumor-infiltrating immune cells in breast

cancer. In the analysis of the correlation between the immune checkpoints and the prognostic LRGs, the ggplot2 R package was selected to perform. As for the analysis of the tumor mutation burden (TMB) and microsatellite instability (MSI), we calculated the correlation score by spearman's correlation analysis, when the *p*-value was less than 0.05, the results were considered as statistically significant.

The clusters analysis of LRGs

The raw counts of RNA-sequencing data (level 3) and corresponding clinical information of breast cancer were obtained from The Cancer Genome Atlas (TCGA), in which the method of acquisition and application complied with the guidelines and policies. Use the R software package ConsensusClusterPlus (v1.54.0) for consistency analysis[27], and use the R software package pheatmap (v1.0.12) for clustering heatmaps. The gene expression heatmap retains genes with SD > 0.1. If the number of input genes is more than 1000, it will extract the top 25% genes after sorting the SD. Limma package (version: 3.40.2) of R software was used to study the differential expression of mRNAs. The adjusted P-value was analyzed to correct for false-positive results in TCGA or GTEx. The results of "Adjusted P < 0.05 and Log (Fold Change) >1 or Log (Fold Change)< − 1" were defined as the thresholds for the screening of differential expression of mRNAs. Volcano plots were constructed using

fold-change values and adjusted P values. The red point in the plot represents the over-expressed mRNAs and the blue point indicates the down-expressed mRNAs with statistical significance. Hierarchical clustering analysis of mRNAs, which were differentially expressed between tumor and normal tissues.

All the above analysis methods and R package were implemented by R foundation for statistical computing (2020) version 4.0.3.

Results

1 Detecting the expression of LRGs in breast cancer.

The expression of the 16 LRGs in breast cancer and normal breast tissues was first detected by data from TCGA-breast cancer. A total of two genes were with no significant change in breast cancer (Fig. 1). More specially, the expression of FABP5, FABP7, FABP3, FABP6, NDUFAB1, FABP2, FABP1, KLF5, LPN2, LPN1, LPN3, EP300 was up-regulated compared with normal tissues, while the expression of FABP4, FABP9, KLF4 was down-regulated (all $p<0.05$)

2 Functional enrichment analysis of LRGs

The protein-protein interaction(PPI) network was constructed to explore the correlation of these LRGs which set with a minimum interaction score of 0.9, as shown in Table1, the analysis results revealed that EP300,

FABP1, FABP2, FABP4, FABP7, KLF4, KLF5 were hub genes (Fig. 2A). GO and KEGG databases were selected to screen the function of the LRGs. We found that these 16 LRGs were mainly involved in the positive regulation of triglyceride metabolic process, acylglycerol metabolic process, neutral lipid metabolic process, triglyceride catabolic process, fatty acid binding, monocarboxylic acid binding, carboxylic acid binding, organic acid binding in GO analysis. Moreover, the results of KEGG pathway analysis revealed that 16 LRGs were mainly involved in the PPAR signaling pathway, Glycerolipid metabolism, Glycerophospholipid metabolism, mTOR signaling pathway (Fig. 2B，Table 2).

3 Construction of the prognostic gene model

For clarifying the prognostic value of these LRGs, Univariate Cox regression analysis was selected to construct a prognostic gene model. The results showed that two genes of LRGs in total were identified with prognostic value and the Kaplan–Meier survival curves were shown in Fig. 3. The prognostic analysis results suggested a poor survival possibility in breast cancer patients with down-regulation of FABP7 (Fig. 3A, p = 0.001) and the up-regulation of NDUFAB1(Fig.3B, p= 0.011). Based on the prognostic value of FABP7 and NDUFAB1 in breast cancer, we constructed a prognostic gene signature containing these two LRGs (FABP7, NDUFAB1) by LASSO Cox regression analysis (Fig. 4A, B),

and the final results were calculated with the formula of the risk score = (-0.1013) * FABP7 + (0.3367) * NDUFAB1. Based on the risk score, two groups were separated into the high-risk and the low-risk. The risk score distribution, survival status, and the expression of these two genes were present. With the risk score increasing, the death risk of breast cancer patients increased, while the survival time decreased, the results shown in Fig. 4C. By the Kaplan–Meier survival curves, we illustrated that the overall survival (OS) rate was poor in the breast cancer patients with high-risk scores(median time=9.5 years) than those with low-risk scores (median time =10.8 years) (p = 0.0053), followed with the AUCs of 0.596, 0.591, and 0.608 in the 1- year, 3-year, and 5-year ROC curves, respectively (Fig. 4D, 4E).

4. The construction of the predictive nomogram

Allowing for the correlation between pathological features and these two prognostic LRGs (FABP7, NDUFAB1), a predictive nomogram was subsequently built to predict the survival probability. By analyzing results, the univariate analyses identified the expression of FABP7 and NDUFAB1, as well as the stage of pT, pN, and pM were the factors that could affect the prognosis of breast cancer patients. More interestingly, the univariate and multivariate analyses illustrated that age was the factor

in affecting the prognosis, the results were shown in Fig. 5A-B. From analyzing the expression of FABP7 and NDUFAB1 with the clinical characteristics, we could see that the expression of FABP7, as well as NDUFAB1, was significantly correlated with the T stage and age, the results were shown in Table 3，4. Furthermore, by the analyzing results of the predictive nomogram, the 3-year and 5-year overall survival (OS) possibility could be predicted relatively well in the entire cohort, as shown in Fig. 5C, D.

5. LRGs were associated with tumor immune infiltration in breast cancer

In our study, we also performed the correlation analysis for the expression of prognostic LRGs (FABP7, NDUFAB1) and immune-cell infiltration in breast cancer using the ssGSEA package in R software. The data demonstrated a significant correlation between the expression of prognostic LRGs (FABP7, NDUFAB1) and the abundance of immune infiltration cells such as CD8+T cells, macrophages, neutrophils, Cytotoxic cells, Eosinophils, NK cells, and Treg cells (Fig.6 A B, all $p<0.05$). This evidence suggested a significant correlation between the prognostic LRGs and the tumor immune infiltration. Moreover, we detected the correlation between the immune checkpoints (TIGIT, PDCD1, CD274, LAG3, CTLA4) and the prognostic LRGs by ggplot2 R

package, the results revealed a significant correlation between the immune-checkpoints and the two prognostic LRGs.(Fig. 7 A B, all $p<0.05$).

6. TMB and MSI analysis of LRGs

Tumor mutation burden (TMB), as well as Microsatellite instability (MSI) analysis, could be used to predict the efficacy of immunotherapy for breast cancer. To clarify whether these two prognostic LRGs could serve as biomarkers for immunotherapy, we analyzed the correlation between the two prognostic LRGs and TMB as well as MSI in breast cancer. The results revealed a positive correlation between TMB and FABP7 (Fig. 8A, p = 2.26e−06) and NDUFAB1 (Fig. 8B, p=0.005). The results revealed that the prognostic LRGs were significantly correlated with tumor immune infiltration and could serve as the biomarkers of immunotherapies for breast cancer.

7 Tumor immune infiltration analysis of the prognostic gene model

To further screen the correlation of the gene prognostic model containing

the prognostic LRGs (FABP7 and NDUFAB1) with the tumor immune microenvironment (TIME) in breast cancer, the ssGSEA method was selected to perform the immune infiltration analysis of this prognostic signature. The analysis results illustrated a negative correlation between the prognostic model containing FABP7 and NDUFAB1(Fig.9). The above results revealed a significant correlation of the gene signature with the TIME in breast cancer.

8. Consensus clustering analysis of LRGs in breast cancer

For exploring the different functions of these 16 LRGs, raw counts of RNA-sequencing data (level 3) and corresponding clinical information from breast cancer were obtained from The Cancer Genome Atlas (TCGA), in which the method of acquisition and application complied with the guidelines and policies. Delta area curve of consensus clustering (Fig.10A)， indicating the relative change in area under the cumulative distribution function (CDF) curve for each category number k compared with k–1. consistency analysis (Fig.10B), the final number of clusters is two (Fig.10C, D)

9 Functional enrichment analysis of the consensus clusters

Volcano plots were constructed using fold-change values and adjusted *p*-value, The red point in the plot represents the over-expressed mRNAs

and the blue point indicates the down-expressed mRNAs with statistical significance, the results revealed that 846 mRNAs were up and 255 mRNAs were down. (Fig.11A). Hierarchical clustering analysis of mRNAs, which were differentially expressed between tumor and normal tissues. (Fig.11B)

To further confirm the underlying function of potential targets of these two clusters in breast cancer, the data were analyzed by functional enrichment analysis performed with GO/KEGG methods, and the results with p <0.05 or FDR <0.05 were considered to be enriched to a meaningful pathway. The analysis results suggested that the up consensus cluster mRNAs were related to the function of oncogenesis and energy metabolic reprogramming of breast cancer, such as PI3K−Akt signaling pathway, MAPK signaling pathway, Insulin secretion, positive regulation of protein kinase B signaling, regulation of hormone secretion, regulation of insulin secretion, reproductive system development(Fig.11C), meanwhile, the down cluster was closely related to immune-related function and signaling pathway, such as p53 signaling pathway, IL−17 signaling pathway, Cell cycle, Chemokine signaling pathway, lymphocyte chemotaxis (Fig.11D). More interestingly, both the consensus clusters were involved in the Estrogen signaling pathway, which is the important signaling pathway for breast cancer.

The above results showed the different function and signature pathways of the consensus clusters of LRGs in breast cancer, which may suggest the clues that the process of lipid metabolic system comprises of the lipid formation and dynamics were related to different biological functions in breast cancer.

Discussion

Breast cancers including different subtypes have been always threatening the health of women worldwide, especially triple-negative breast cancer (TNBC) due to the lack of effective therapeutic targets[28]. Studies have proven that breast cancer cells can express naturally processed and demonstrated unique extraordinary mutations that could be able to be recognized by the immune system of patients[29]. Therefore, immunotherapies such as immune checkpoint blockades and others have offered new clinical strategies for breast cancer patients. However, given that only a few breast cancer patients have exhibited high immune infiltration and valid responses to immunotherapies. Hence, there is a huge demand for the identification of novel potential effective immunotherapeutic targets from the new crosstalk of tumor cells in breast cancer.

Cancer cells have been known to be able to take advantage of the altered metabolic community to maintain their survival, proliferation, and cancer progression[30-32]. The altered lipid metabolic process of cancer cells comprises of the fatty transport, formation, binding, and dynamics can further reprogram and impacts other cells in the tumor microenvironment (TME) which contributes to regulate the processes of the oncogenesis, aggravation, metastasis, and recurrence of breast cancer[33,34]. Numerous studies of the TME have redefined the tumors from the simple gatherings of tumor cells to a complicated community which are composed of not only tumor cells but the immune cells, fibroblasts, vascular endothelial cells, and other stromal cells surrounded[35]. The tumor immune microenvironment (TIME), which represents the immune components of the TME, can both promote and inhibit the behavior and communication of tumor cells[36,37]. TIME has been proven to be playing a critical role in the potential immunotherapeutic targets[38]. Therefore, characterizing the sophisticated lipid metabolic bi-directional interplay between tumor cells and the TIME may reveal key vulnerabilities of breast cancer and identify novel potential biomarkers for immunotherapeutic strategies.

In this study, we first evaluated the expression and prognostic value of these 16 lipid metabolic-related genes (LRGs) in breast cancer, and the results showed that the expression of FABP5, FABP7, FABP3, FABP6, NDUFAB1, FABP2, FABP1, KLF5, LPN2, LPN1, LPN3, EP300 was

increased compared with normal tissues, meanwhile the expression of FABP4, FABP9, KLF4 was decreased. Prognosis analysis results identified a poor survival rate in breast cancer patients with low expression of FABP7 and high NDUFAB1 expression. GO/KEGG function enrichment analysis was then performed, and we illustrated that these 16 LRGs were mainly involved in the lipid metabolic reprogram activities such as triglyceride metabolic process, acylglycerol metabolic process, fatty acid binding, PPAR signaling pathway, and mTOR signaling pathway which are related to oncogenesis, progress and inflammation of breast cancer. The results suggested these LRGs have a significant correlation with the carcinogenesis, aggravation, metastasis, and recurrence process of breast cancer[39-41].

To further clarify the prognostic value of these LRGs, the Kaplan-Meier method was selected and the results suggested a poor survival rate in breast cancer patients with low expression of FABP7 and the high expression of NDUFAB1. Based on the prognostic value of FABP7 and NDUFAB1, the LASSO Cox regression analysis was performed to construct a prognostic gene model, which could predict the overall survival (OS) rate of breast cancer patients with medium-to-high accuracy, the final signature was calculated with the formula of the risk score = (-0.1013) * FABP7 + (0.3367) * NDUFAB1. Based on the risk score, two groups were separated into the high-risk and the low-risk and the

Kaplan–Meier survival curves revealed that the breast cancer patients with high-risk scores had a worse overall survival (OS) rate than those with low-risk scores. A predictive nomogram was subsequently constructed to compare the correlation of the pathological features with these two prognostic LRGs (FABP7, NDUFAB1) in breast cancer patients and the results revealed that the FABP7, NDUFAB1 and pT stage, pN stage, and pM stage were the factors that could affect the prognosis of breast cancer patients, moreover, the 3-year and 5-year overall survival rates could be predicted relatively well compared with an ideal model in the entire cohort.

Tumor mutation burden (TMB) [42], as well as Microsatellite instability (MSI) [43]analysis, could be used to predict the efficacy of immunotherapy for breast cancer. By the TMB, MSI, tumor immune-cell infiltration, and immune-checkpoints analysis, we illustrated that FABP7 and NDUFAB1 were significantly correlated with tumor immune infiltration, which suggested a correlation with the tumor immune microenvironment (TIME), The results of the TMB and MSI analysis revealed FABP7 and NDUFAB1 could predict the efficacy and serve as predictive biomarkers for breast cancer immunotherapy. More interestingly, by the immune cells infiltration analysis of the prognostic signature constructed by FABP7 and NADUFAB1, we could see a more significant correlation with the tumor immune cells which suggested the impact of the signature on the

TIME of breast cancer.

Another important finding of our study revealed the difference in the function of these 16 LRGs by consistency analysis. To further confirm the underlying function of potential targets of these two consensus clusters in breast cancer, the data were analyzed by functional enrichment using the GO and KEGG databases. The analysis results suggested that the up cluster was significantly related to PI3K−Akt signaling pathway, MAPK signaling pathway, Insulin secretion, positive regulation of protein kinase B signaling, regulation of hormone secretion, regulation of insulin secretion, reproductive system development, which was closely related to the function of oncogenesis and metabolic reprogram process of breast cancer[44-46]. Meanwhile, the down consensus cluster was closely related to immune-related function and signaling pathway, such as p53 signaling pathway, IL−17 signaling pathway, Cell cycle, Chemokine signaling pathway, lymphocyte chemotaxis[47,48]. More interestingly, both the consensus clusters are involved in the Estrogen signaling pathway, which is the important signaling pathway for breast cancer[49,50]. Studies have proven that the disturbances in the lipid metabolic system could lead to the unbalanced distribution of nutrients between tumor cells and immune cells in the tumor microenvironment (TME)[51]. The above results showed the different function and signature pathways of the consensus clusters of LRGs in breast cancer, which suggested that the lipid metabolic system

was significantly related to the oncogenesis and process of breast cancer. The results also revealed that the lipid metabolic system was associated with the immune components of the tumor microenvironment in the reassignment of the nutrients which may be induced by the estrogen signaling pathway.

In conclusion, a comprehensive analysis was performed to evaluate the part of the lipid metabolic system and identified a prognostic signature containing two genes(FABP7 and NDUFAB1) for immunotherapies in breast cancer. Our results also revealed new evidence of the vulnerabilities in the bidirectional interplay between the lipid metabolic system and the tumor immune microenvironment (TIME) which may contribute to deciphering the heterogeneity of the TIME in breast cancer. Although there is still much to decipher the complicated interplay between the lipid metabolic system and the TIME, with the development of novel technologies such as immunogenomics, single-cell, and artificial intelligence, the components and novel crosstalk between tumor cells and immune cells could be better revealed and understand for immunotherapies in breast cancer.

Data statement:

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

Acknowledgment:

The authors express thanks to teachers and students for their help

Funding:

This work was supported by the National Natural Science Foundation of China (81702593)

Ethics statement

This study was approved by the Ethics committee of the First Affiliated Hospital of China Medical University (Approval number:AF-SOP-07-1.1-01).

Author contributions:

All authors participated in the analysis of the data, drafted or revised the article, gave final approval to the version to be published, and agreed to take responsibility for all aspects of the work.

Footnote:

The authors report no conflicts of interest in this work.

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Figures

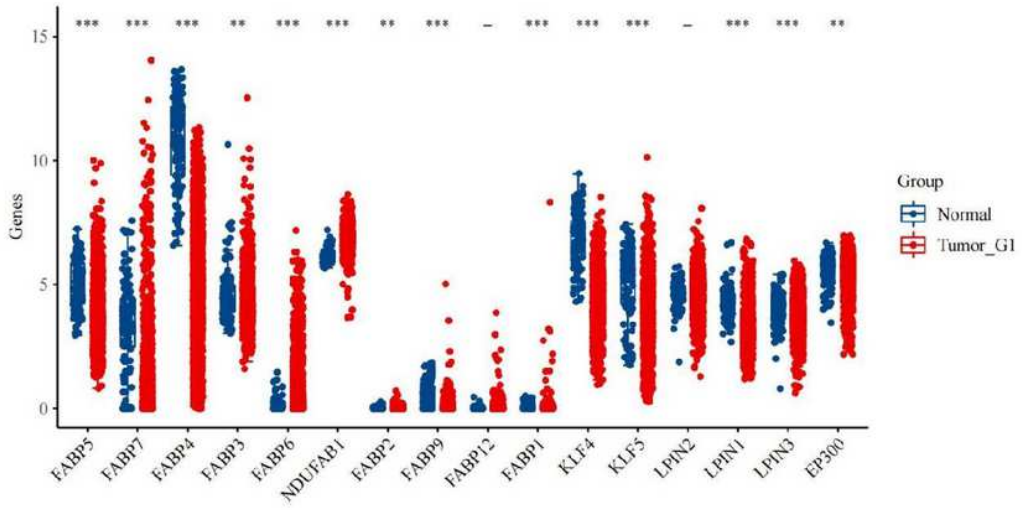

Figure 1

The expression of 16 LRGs in breast cancer and breast tissues, Tumor, red; Normal, blue. LRGs, lipid metabolic related genes.

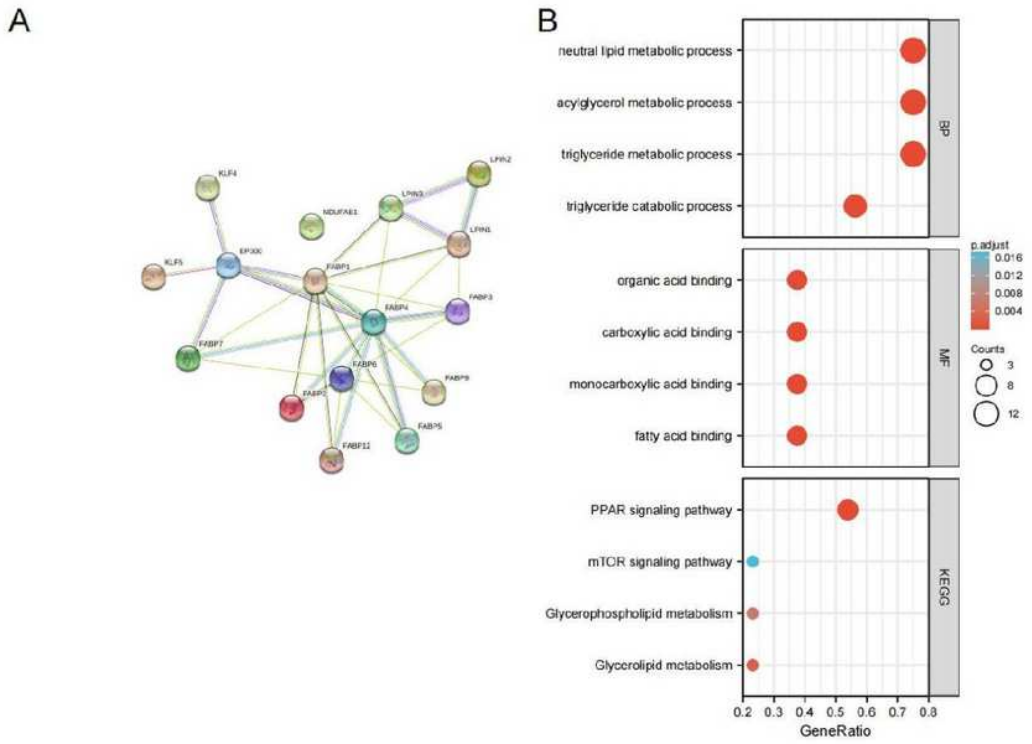

Figure 2

The functional enrichment analysis of LRGs in breast cancer. A The PPI network of LRGs using STRING database. B The enriched item in gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. The size of circles represented the number of genes enriched. PPI, protein-protein interaction; BP, biological process; MF, molecular function.

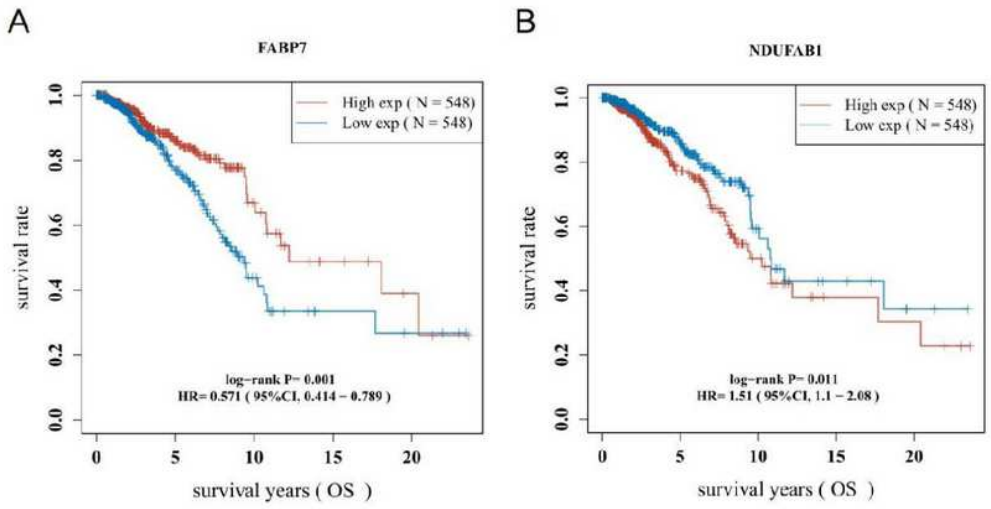

Figure 3

The prognostic value of LRGs in breast cancer. The overall survival curve of A FABP7 B NDUFAB1 in breast cancer patients in the high-/low-expression group.

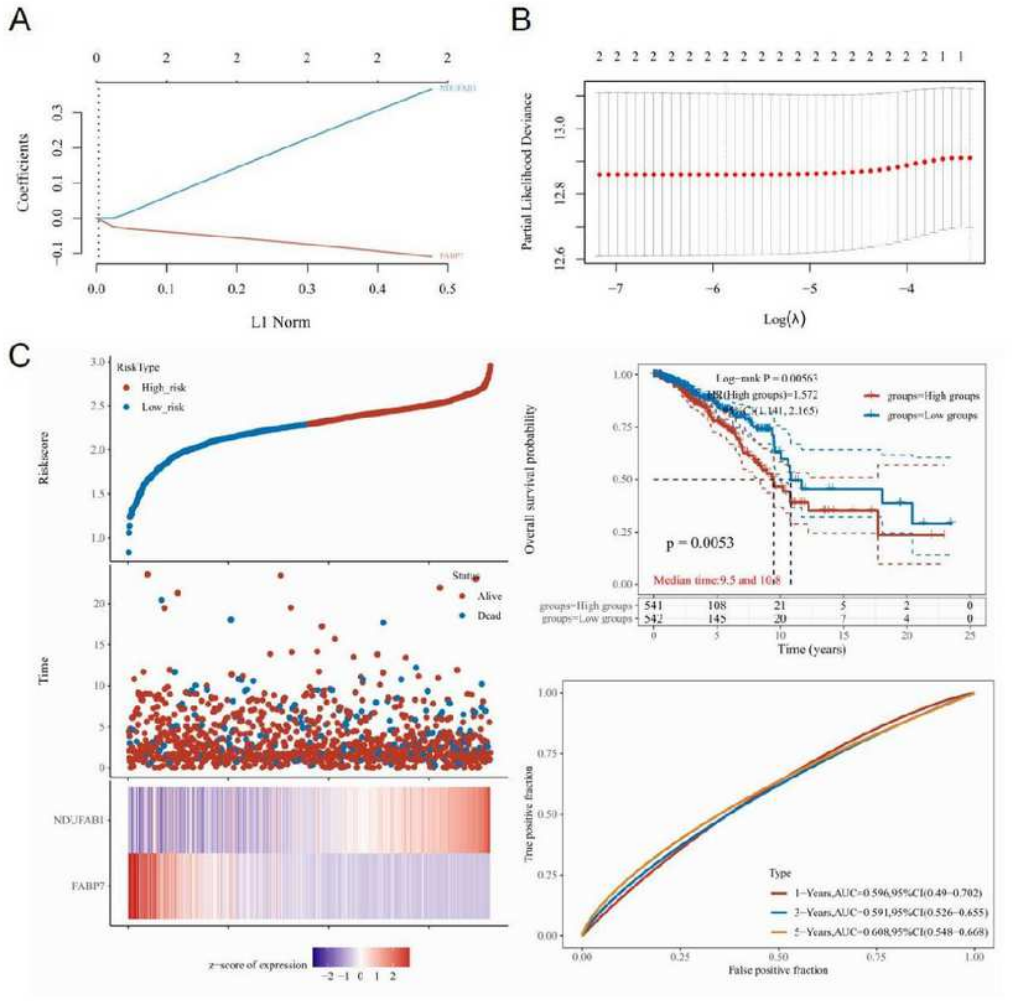

Figure 4

Construction of a prognostic LRGs model. A LASSO coefficient profiles of the two LRGs. B Plots of the ten-fold cross-validation error rates. C Distribution of risk score, survival status, and the expression of 2 prognostic LRGs in breast cancer. D-E Overall survival curves for breast cancer patients in the high-/low-risk group and the ROC curve of measuring the predictive value.

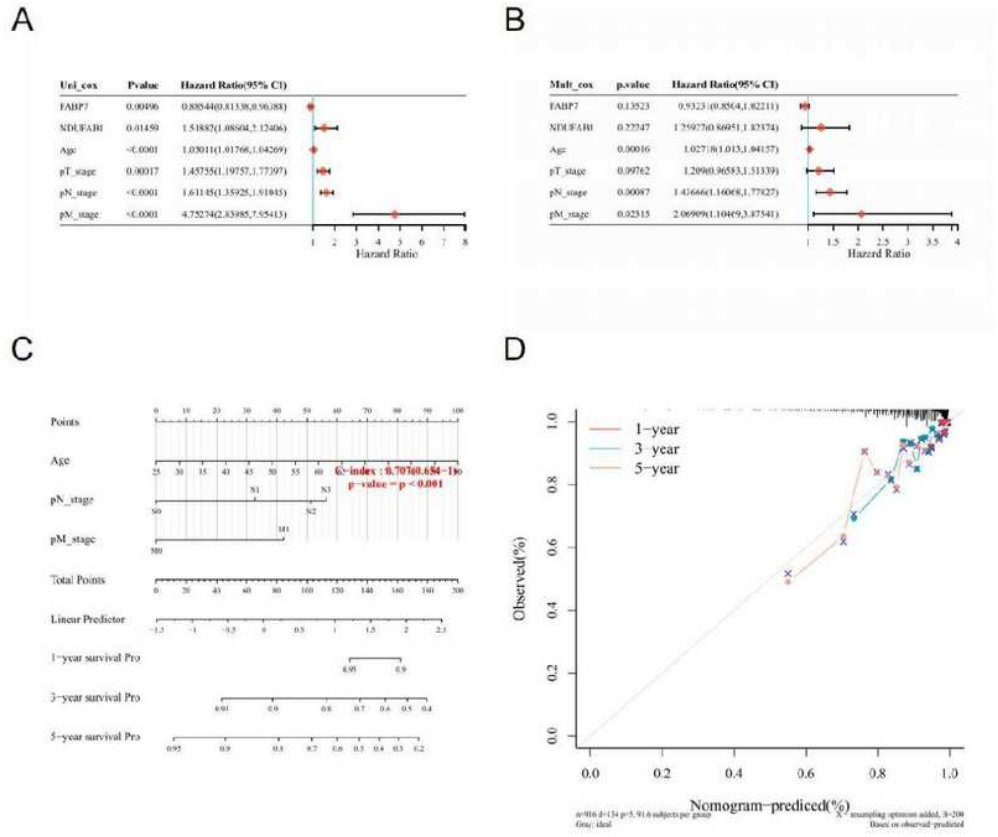

Figure 5

Construction of a predictive nomogram. A-B Hazard ratio and p□value of the constituents involved in univariate and multivariate Cox regression considering clinical the parameters and two prognostic LRGs in breast cancer. C-D Nomogram to predict the 1-year, 3-year, and 5-year overall survival rate.

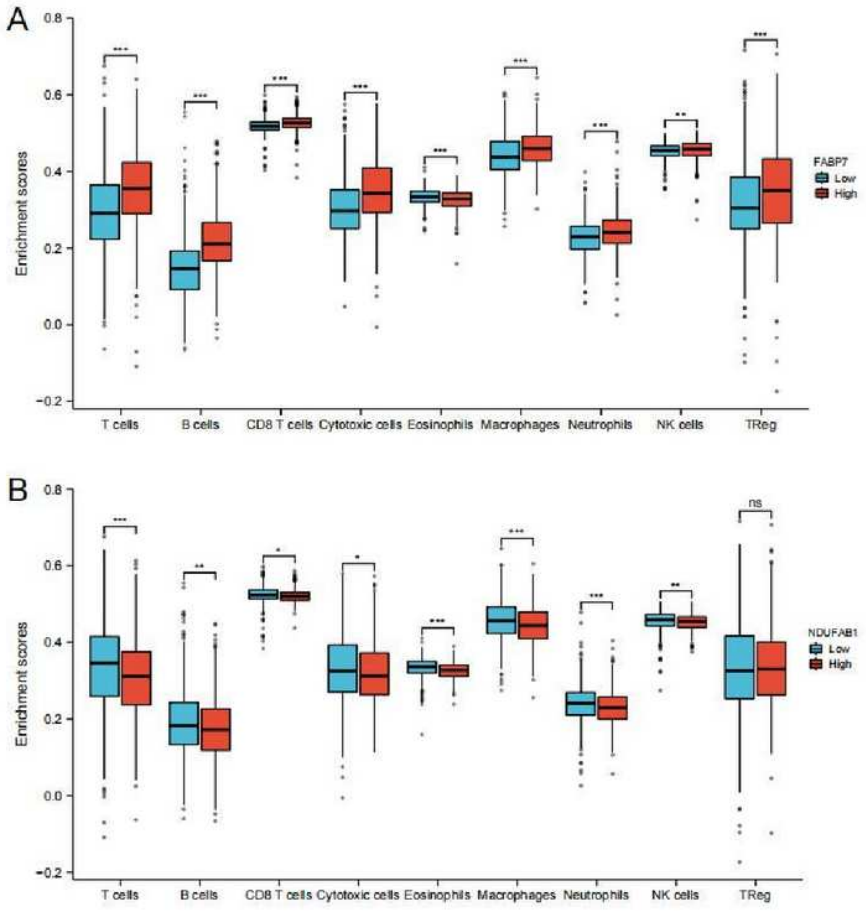

Figure 6

Fig.6 The immune-cell infiltration analysis of the two prognostic LRGs. A-B The association between the abundance of immune cells and the expression of FABP7, NDUFAB1 in breast cancer. Asterisks represent levels of significance *p < 0.05, **p < 0.01, ***p < 0.01.

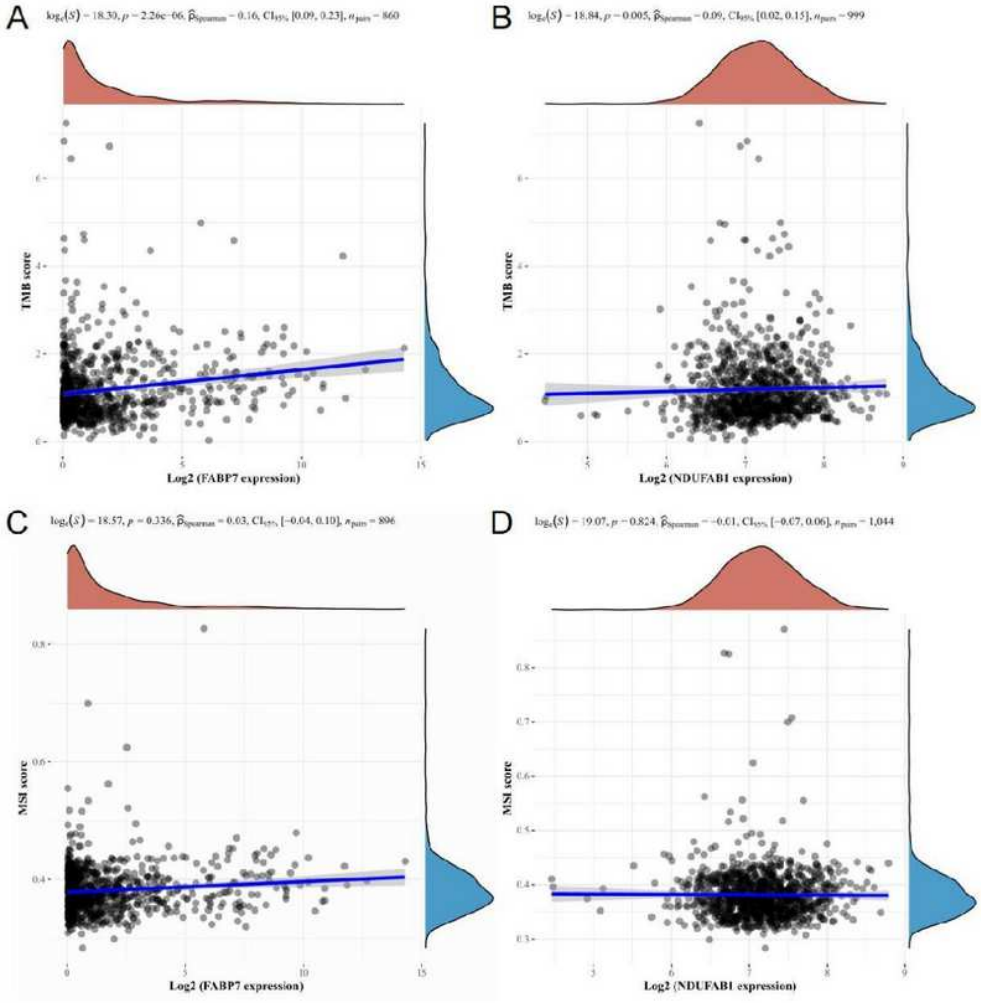

Figure 7

TMB, MSI analysis of the prognostic LRGs (FABP7 and NDUFAB1) in breast cancer. A-B The correlation between two prognostic LRGs and TMB in breast cancer. C-D The correlation between two prognostic LRGs and MSI in breast cancer. TMB, tumor mutation burden; MSI, microsatellite instability.

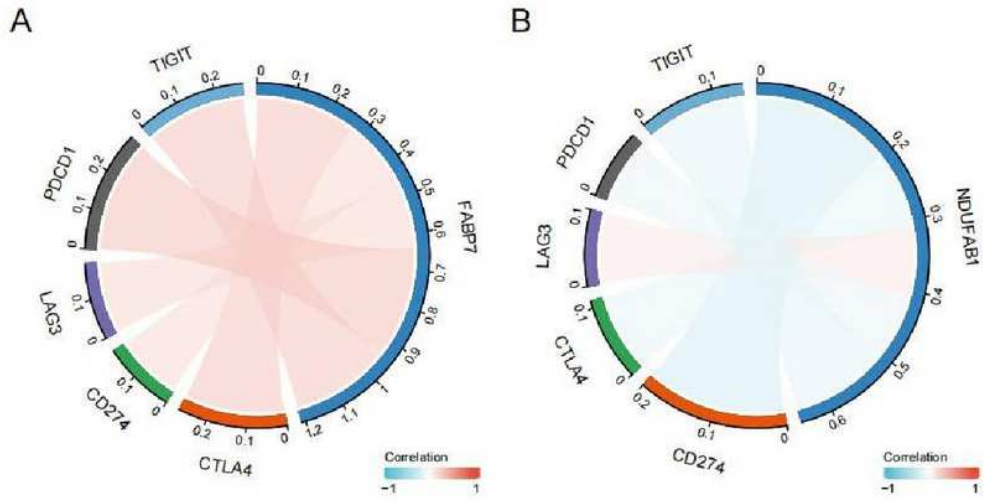

Figure 8

The correlation of the immune-checkpoints(TIGIT, PDCD1, LAG3, CD274, CTLA4) with A, FABP7; B, NDUFAB1, all p<0.05.

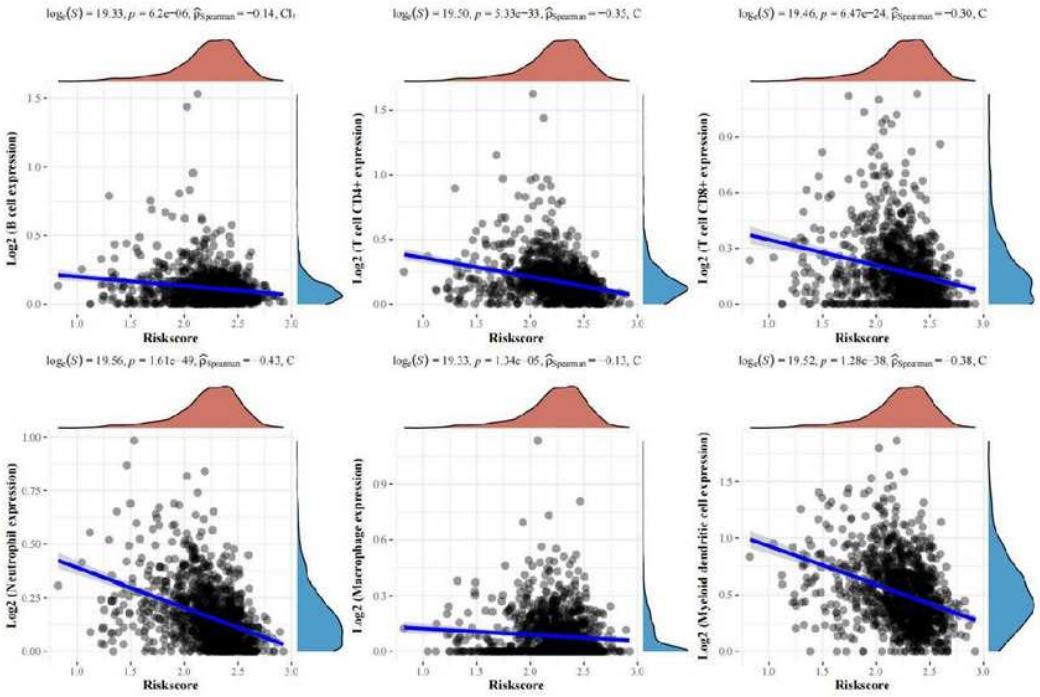

Figure 9

Immune-cell infiltration analysis of the prognostic signature containing two prognostic LRGs (FABP7 and NDUFAB1) .

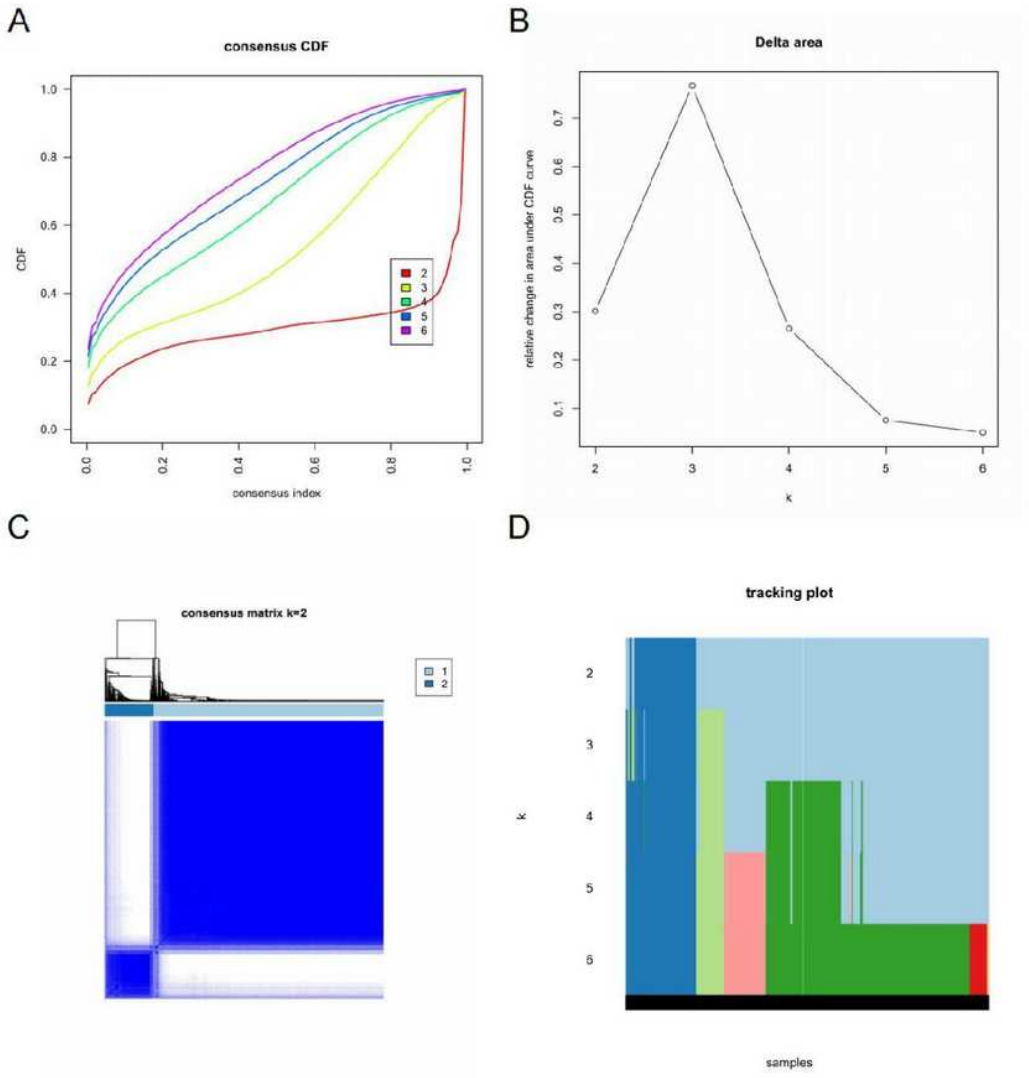

Figure 10

Consensus Clustering Analysis of lipid metabolic-related gene clusters A-B Cumulative distribution function (CDF) of consensus clustering by consistency analysis; C-D Consensus matrices of the sarcoma patients for k = 2.

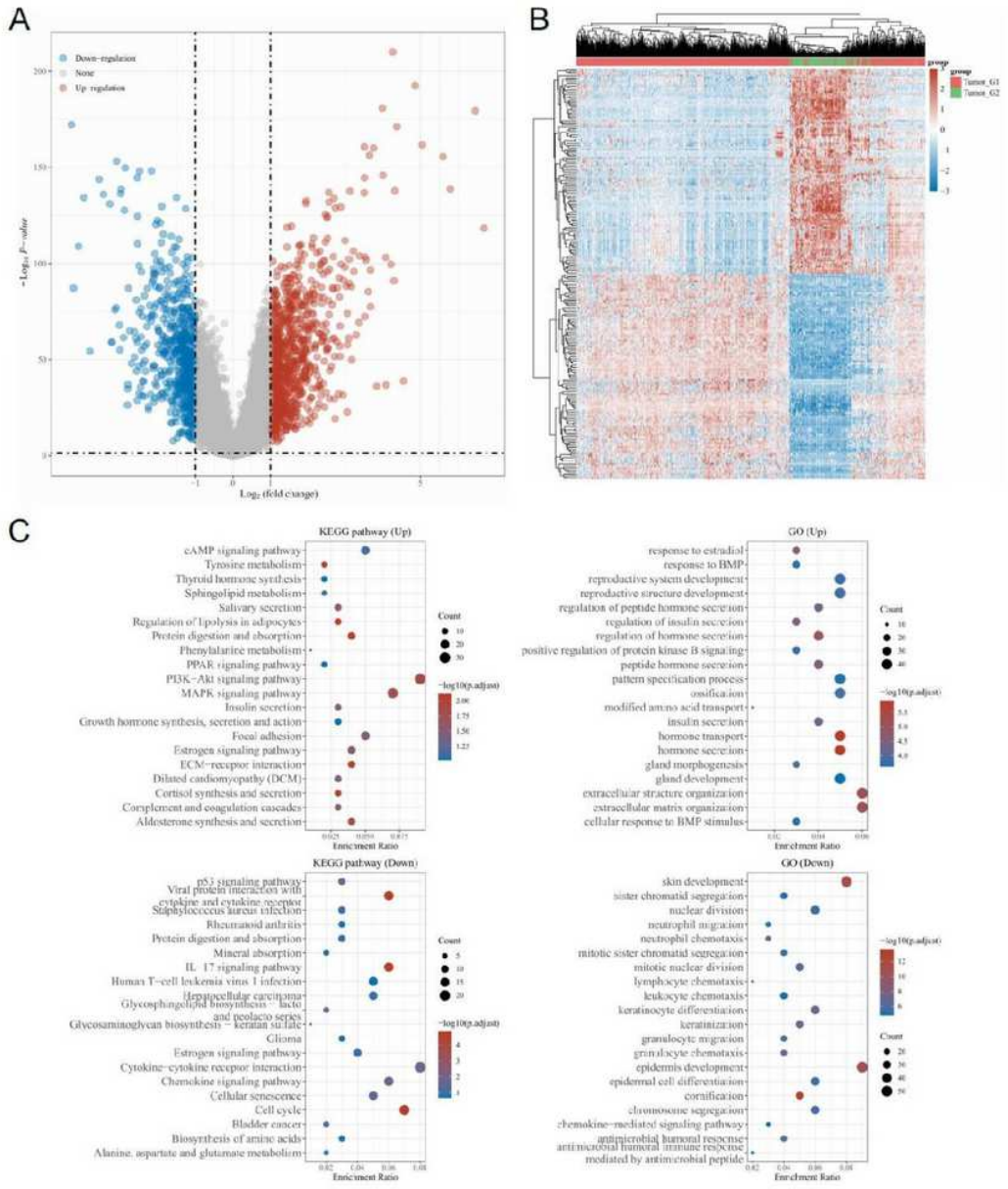

Figure 11

A,Volcano plots of clustering analysis of mRNAs, B, Hierarchical clustering analysis of mRNAs, C-D The enriched item in gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of consensus clusters. The size of circles represented the number of genes enriched.

Supplementary Files

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- Table.pdf