Original Article

A comprehensive analysis of the expression and regulation network of lymphocyte-specific protein tyrosine kinase in breast cancer

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Background: Lymphocyte-specific protein tyrosine kinase (*LCK*), an encoded Src family protein tyrosine kinase, performs a pivotal molecular signaling role in the selection and maturation processes during T-cell development. Although aberrant LCK expression is known to have a significant association with carcinogenesis, the underlying role of *LCK* in breast cancer (BC) is still obscure.

Methods: An analysis of the levels of *LCK* mRNA expression in BC was performed, and the value of *LCK* expression for predicting the prognosis of patients with BC was studied using various online data resources, which included Oncomine, Gene Expression Profiling Interactive Analysis (GEPIA), and UALCAN. The web-based NetworkAnalyst tool was utilized to investigate the functional network of differentially expressed *LCK*. LinkedOmics was employed to identify the genes with which *LCK* has correlations in BC, together with the kinases, microRNAs, and transcription factors (TFs) potentially targeted by *LCK* in BC. The expression levels of LCK and its significantly correlated genes in BC were investigated with the Human Protein Atlas (HPA).

Results: We observed a significant difference in the level of LCK mRNA expression between BC patients and healthy individuals, and a higher LCK expression was associated with poor overall survival (OS). The functional enrichment results revealed that the differential expression of *LCK* was mainly involved in the regulation of immune response and inflammatory response in BC. The expression of significantly related genes, such as *inducible T-cell kinase (ITK)*, *CD5*, *CD96*, *CD247*, *SH2 domain containing 1A (SH2D1A)*, *phosphatidylinositol-4,5-bisphosphate 3-kinase (PIK3CD)*, *Src-like-adaptor 2 (SLA2)*, *and interleukin 2 receptor (IL2RG)*, was associated with poor OS in patients with BC. Regulatory network analysis found that LCK regulated immune cells, cancer progression, apoptosis, and cell cycle signal transduction through cancer-related kinases (ITK and MAPK3), miRNAs (miR-345 and miR-524), and TFs (AP1, SRF, and E2F1).

Conclusions: This study presents new perspectives on the differential expression and prognostic value of *LCK* in BC. Our observations will provide a basis for further study on the oncogenic and regulatory roles of LCK in BC.

Keywords: Breast cancer (BC); bioinformatics analysis; functional network; lymphocyte-specific protein tyrosine kinase (*LCK*); prognosis

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Introduction

Breast cancer (BC) was the one of the commonest cancer types for China (1). Each year, almost 1.7 million cases of BC are diagnosed, and 500,000 people die due to this disease (2). Approximately 1 in every 8 to 10 women will suffer from BC in their lifetime (3). With the development of advanced diagnostic methods and more effective therapies in the past few years, mortality among patients with BC has decreased. In 2016, BC was responsible for 8% of all deaths in the European Union (4), and it is still the biggest contributor to cancer-related death around the world. Therefore, it is crucial to find a more sensitive and effective biomarker for the diagnosis of BC, as well as potential therapeutic targets.

Lymphocyte-specific protein tyrosine kinase (LCK) was first identified to be part of the Src protein tyrosine kinase family in the 1980s (5). LCK is composed of N-terminal site (Src-homology 4, SH4 domain), a unique region, a SH3 and SH2 domain, a catalytic domain, and a short C-terminal tail (6). Later research demonstrated LCK to be a regulator of T-cell receptor (TCR) signaling, and T-cell development and homeostasis. LCK also acts as an important regulator of chimeric antigen receptor (CAR)-engineered T cells (7). Further studies showed that LCK is differentially expressed in various types of cancers and that the biological function of LCK differs depending on the cancer. Cancers in which LCK expression has been detected include BC, lung cancer, colon cancer, cholangiocarcinoma, and glioma (8-12). In cholangiocarcinoma, high LCK expression is considered to be a risk factor for tumor recurrence (8). Previous studies have also reported that LCK may be an important regulator of cancer stem cells (CSC) (13), which contribute to tumor drug resistance and recurrence. In another study, LCK is a potential therapeutic gene for acute myeloid leukemia (AML) (14). Compared to Bai *et al.* study, we not only analyzed the LCK gene but also explored the significant correlated-genes and significant regulators such as PIK3CD, SLA2, IL2RG, ITK, MAPK3, miR-345 and miR-524 of LCK (15). Based on the evidence described above, it can be speculated that LCK may be a diagnostic biomarker and a potential therapeutic target for patients with cancer. However, a lack of clarity still surrounds the role LCK plays in BC. Here, we studied the expression levels and prognostic value of LCK in patients with BC using bioinformatics tools. We mainly used The Cancer Genome Atlas (TCGA) data to perform this study, TGCA is a public funded project that aims to catalogue and discover major cancer-causing genomic alterations to create a comprehensive "atlas" of cancer genomic profiles (16).

We present the following article in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037/tcr-21-328).

Methods

UALCAN (http://ualcan.path.uab.edu) is an online portal which allows users to interactively and straightforwardly conduct comprehensive analysis of gene expression data from The Cancer Genome Atlas (TCGA) (17). Using UALCAN, we examined the expression level of LCK in BC according to different clinicopathological features, such as age, sex, histological subtype, co-mutation status, and stage.

Gene Expression Profiling Interactive Analysis (GEPIA) analysis

GEPIA (http://gepia.cancer-pku.cn) is an online server delivering functions that can be customized by the user to rapidly analyze data from TCGA and Genotype-Tissue Expression (GTEx) projects (18). To study the prognostic roles of LCK and the genes with which it is significantly correlated in BC, we constructed survival curves using GEPIA. Independent-samples *t*-test was employed for the prognostic analysis, with $P<0.05$ set as the threshold for significance.

PrognoScan analysis

PrognoScan is a database that can be used to investigate the associations between defined genes and prognosis for multiple cancers (https://www.abren.net/PrognoScan/) (19). We validated the prognostic role of LCK in BC via PrognoScan with data derived from the Gene Expression Omnibus (GEO).

GeneMANIA analysis

GeneMANIA (https://genemania.org) is website that can be used to construct protein-protein interaction (PPI) networks (20). We use GeneMANIA to predict the function of the LCK gene and visualize the gene network.

LinkedOmics analysis

LinkedOmics (http://www.linkedomics.org) contains

multi-omics and clinical data of 11,158 individuals with 32 different cancers from TCGA. This web-based resource allows researchers to access multi-omics data for analysis and comparison within and among cancer types (21). We found the genes with significant correlations with LCK (Pearson's correlation ≥0.6) in the LinkedOmics dataset and constructed a heatmap of the top 50 correlated genes. Further, the differentially expressed genes within the TCGA BC cohort were analyzed via the "LinkFinder" module. The LinkedOmics database was also used to analyze the possible kinase targets, miRNA target, and transcription factor (TF) targets of LCK in BC.

TIMER analysis

The TIMER (https://cistrome.shinyapps.io/timer) web server can be used to analyze immune cell infiltration comprehensively and systematically in a wide variety of tumors (22). In our study, the TIMER database was utilized for the analysis of immune cell infiltration in BC, and to examine the relationship of immune infiltration with prognosis in patients with BC.

Human Protein Atlas (HPA) analysis

The HPA has the aim of mapping every protein found in the cells, tissues, and organs of humans through the use of integrated omics technologies (http://www.proteinatlas.org) (23). Differences in the expression level of LCK in tumor and normal tissue samples were examined using the HPA.

This study was approved by the Academic Committee of Guangdong Medical University and conducted according to the principles of the Helsinki Declaration (as revised in 2013).

Statistical analysis

The gene expression levels thresholds of |log2 fold change| >1.0 and false discovery rate (FDR <0.05), P value <0.05 seemed as significant difference, the survival analysis P value <0.05 was seemed as the significant influence prognosis, and the spearman correlated value >0.6 seemed significantly correlated.

Results

Expression levels of the LCK gene in patients with BC

To examine the relationship between *LCK* gene expression and BC, UALCAN was used to analyze the level of *LCK* mRNA in BC and normal tissue samples (*Figure 1*). Significant upregulation of *LCK* mRNA was observed in patients with BC. *LCK* expression was found to differ between normal and tumor tissues when data were analyzed according to BC stage, race, sex, age, main subtypes, major subclasses with triple-negative BC, menopausal status, histological subtype, nodal metastasis status, and TP53 mutation status. To summarize, compared to normal samples, BC samples showed a significant increase in the level of *LCK* gene expression.

The relationship of LCK with patient prognosis in BC

To determine whether the *LCK* gene has an influence on survival in BC, we next studied the relationship of LCK expression with the prognostic outcomes of patients with BC using the PrognoScan and GEPIA databases. Data from both databases showed that the expression of LCK significantly impacted the overall survival (OS) of patients with BC. Analysis using PrognoScan revealed an association between a high expression of LCK and a good prognostic outcome (P=0.012) (*Figure 2A*). Similarly, results were observed with GEPIA, which revealed a marginal association between a high level of LCK and poor OS (P=0.041) (*Figure 2B,C*).

Correlated significant genes of LCK and their role in BC

We continued our investigation of the role potentially played by *LCK* in BC by analyzing the mRNA sequencing data of 526 patients with BC from TCGA with the LinkFinder module in LinkedOmics. A volcano plot was generated, which showed significantly positive correlations of LCK with CD5 and CD247, with a false discovery rate (FDR) of <0.01. Heatmaps in *Figure 3A,B,C* show the top 50 most significant genes with a positive or negative association with LCK. We found that CD5 (Spearman correlation =9.166e−01, P=2.249e−149), CD96 (Spearman correlation =9.074e−01, P=2.325e−141), CD247 (Spearman correlation =9.190e−01, P=1.124e−151), IL2RG (Spearman correlation =8.715e−01, P=1.583e−116), ITK (Spearman correlation =8.860e−01, P=1.547e−125), PDCD1 (Spearman correlation =8.594e−01, P=8.146e−110), PIK3CD (Spearman correlation =7.790e−01, P=5.088e−77), SH2D1A (Spearman correlation =9.079e−01, P=8.332e−142), and SLA2 (Spearman correlation =8.677e−01, P=2.359e−114) were strongly associated with LCK in BC (*Figure 4*).

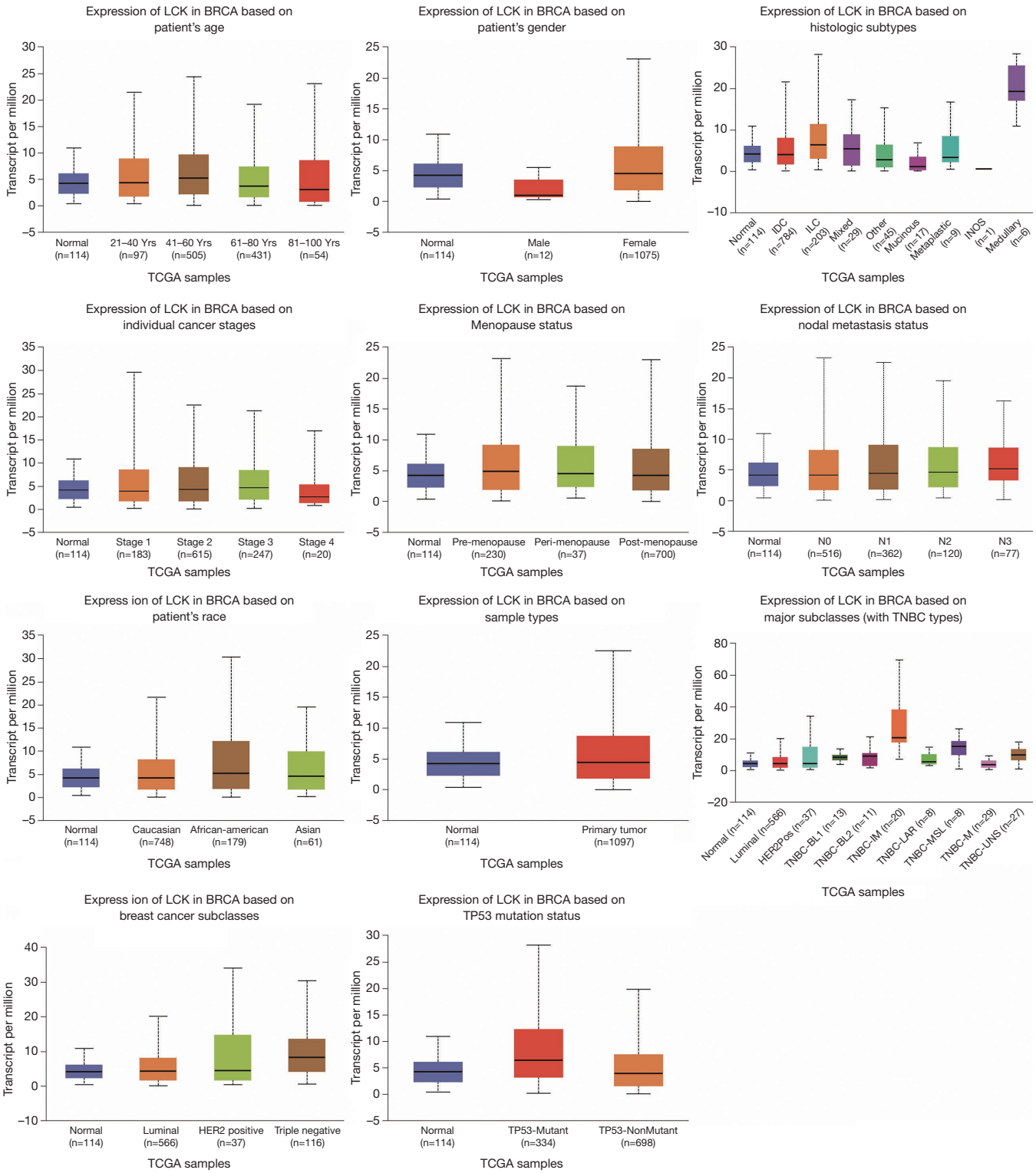


Figure 1 The expression levels of LCK in breast cancer based on different clinical features. LCK, lymphocyte-specific protein tyrosine kinase.

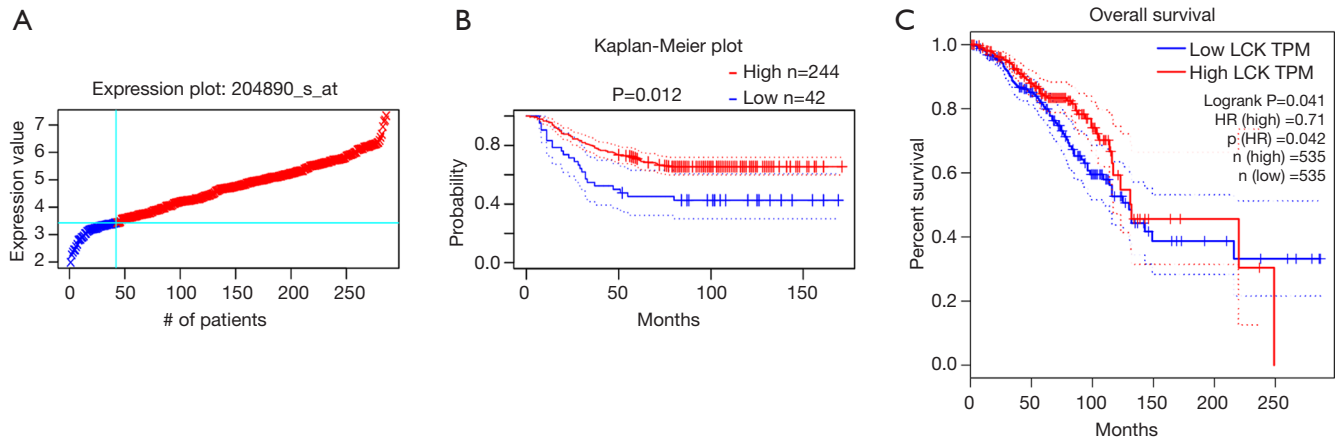


Figure 2 The LCK expression levels and its roles in breast cancer. (A) The LCK expression levels in breast cancer. (B,C) The relationship between LCK expression level and prognostic role for breast cancer. LCK, lymphocyte-specific protein tyrosine kinase.

Next, GEPIA was used to evaluate the prognostic value of the genes positively related to LCK in BC. CD5 (P=0.0037), CD96 (P=0.0036), CD247 (P=0.0058), IL2RG (P=0.0085), ITK (P=0.0019), PDCD1 (P=0.0095), PIK3CD (P=0.022), SH2D1A (P=0.002), and SLA2 (P=0.025) showed significant associations with poor OS in BCs with a high transcriptional level of LCK (*Figure 5*).

Association of LCK expression with immune cell infiltration and prognosis

We further analyzed the association of LCK expression with immune cell infiltration and prognosis in BC using the TIMER database. The following associations were identified: In BCs, tumor purity (P=8.62e−71), B cells (P=2.08e−77), CD8+ T cells (P=5.65e−73), CD4+ T cells (P=3.77e−145), neutrophils (P= 5.43e−104), and dendritic cells (P=3.09e−148); in basal-like BCs, tumor purity (P=3.93e−14), B Cells (P=1.75e−15), CD4+ T cells (P=3.25e−11), neutrophils (P=1.57e−10), and dendritic cells (P=3.36e−14); in human epidermal growth factor receptor (HER2)-positive BCs, tumor purity (P=1.17e−05), CD8+ T cells (P=7.08e−13), CD4+ T cells (P=1.39e−11), neutrophils (P=1.35e−08), and dendritic cells (P=1.60e−10); and in luminal BCs, B cells (P=4.36e−39), CD8+ T Cells (P=1.22e−67), CD4+ T Cells (P=3.89e−85), neutrophils (P=3.05e−57), and dendritic cells (P=1.57e−82); all partial cord >0.5 or <−0.5 show in *Figure 6*. LCK was statistically significantly associated with OS in patients with luminal BCs (P=0.019). In the subgroup analysis, B cells were found to be statistically significant for all BCs (P=0.046) and BCs HER2 (P=0.017), with patients with a high expression of LCK having worse OS than patients with a low expression (*Figure 7*).

Functional enrichment analysis of LCK

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analyses of LCK were performed, and the results are shown in *Figure 8*. LCK was found to be localized in the plasma membrane and pericentrosomal vesicles, and to bind to cell surface receptors, including CD4, CD8, and other signaling molecules. Multiple alternatively spliced variants encoding the same protein have been described. LCK was shown to be mainly involved in lymphocyte-mediated immunity, activation of natural killer cells, regulation of leukocyte activation, and regulation of viral defense.

Kinase and TF targets of LCK

The focus of our analysis next shifted to the kinases, miRNAs, and TFs potentially targeted by LCK in the LinkedOmics database. The most significant kinase targets of LCK were found to be ITK, MAPK3, HCK proto-oncogene (HCK), and protein kinase C theta (PRKCQ) (*Table 1*). The miRNA targets of LCK are shown in *Table 2*. We identified (AGTCAGC) miR-345, (CTTTGTA) miR-524, (CAGCCTC) miR-485-5P, (TTTGCAG) miR-518A-2, (AGGTGCA) miR-500, and (AGCATTA) miR-

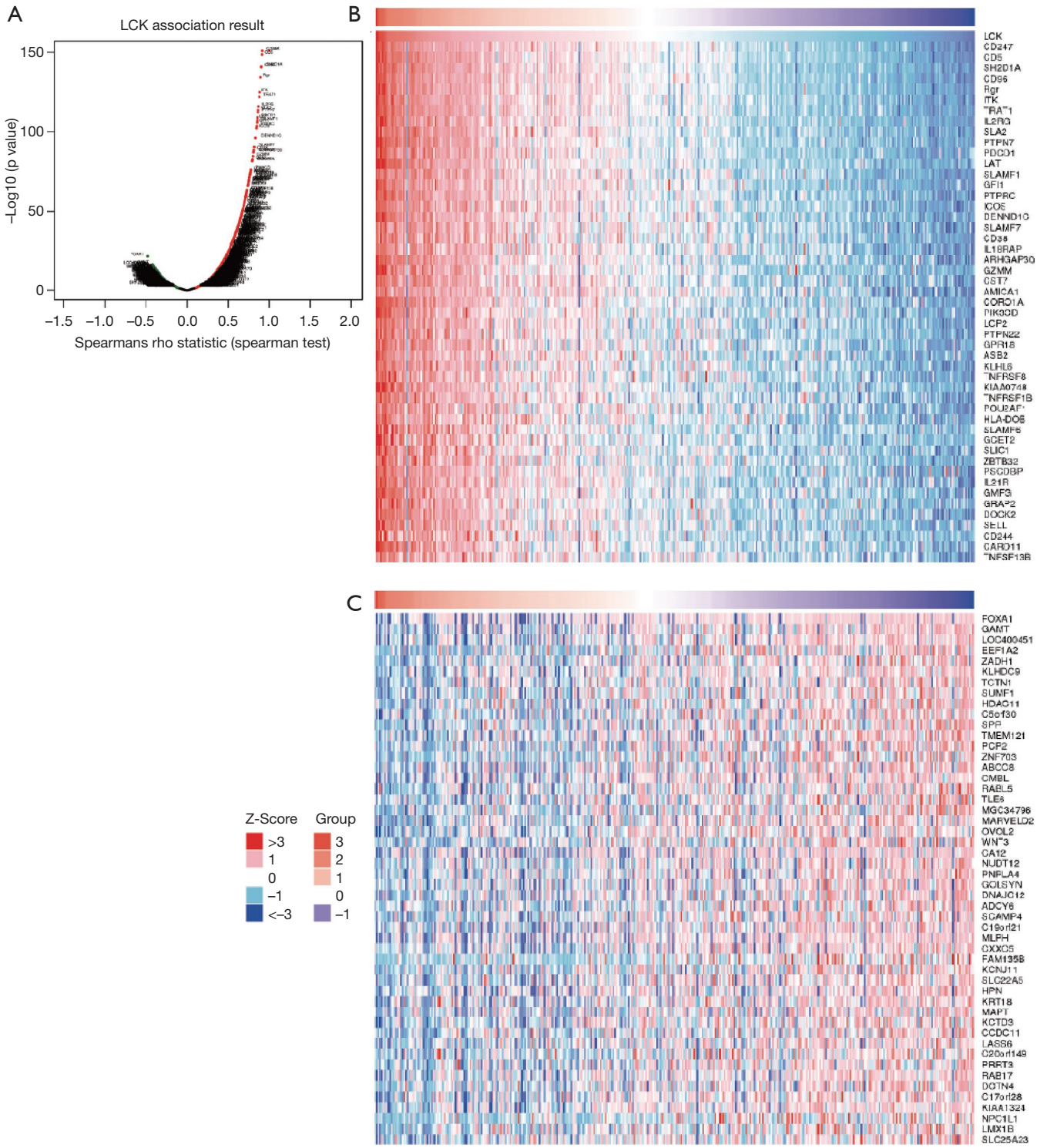


Figure 3 Differentially expressed genes correlated with differentially expressed LCK in breast cancer (LinkedOmics). (A,B,C) Volcano plots and heat maps indicating genes positively and negatively genes correlated with LCK in breast cancer, respectively (top 50). Red suggests positively correlated genes, and green suggests negatively correlated genes. LCK, lymphocyte-specific protein tyrosine kinase.

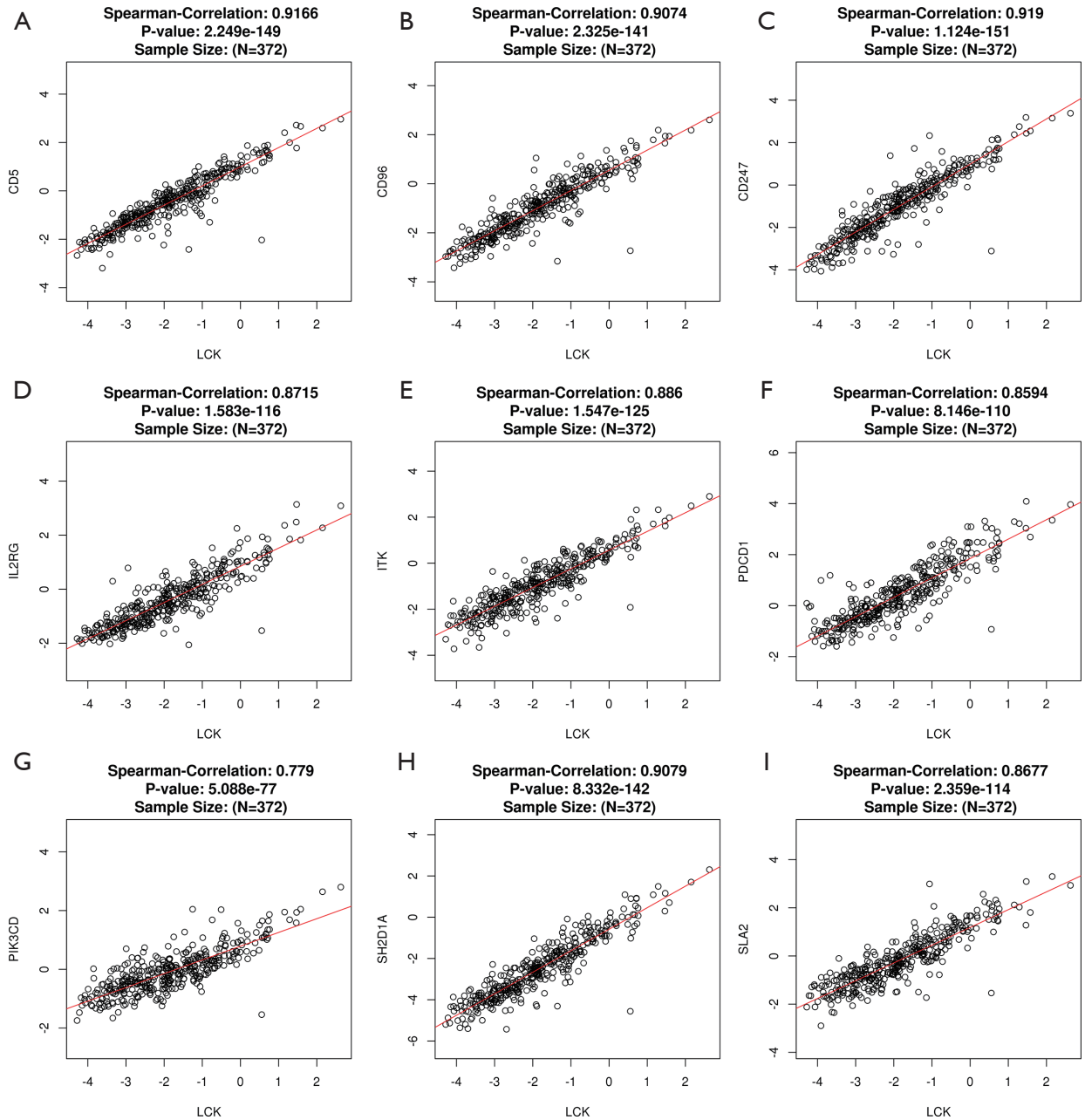


Figure 4 Genes significantly related to LCK in BC via LinkedOmics. (A) The scatter plots show Pearson's correlation of LCK expression with expression of CD5. (B) The scatter plots show Pearson's correlation of LCK expression with expression of CD96. (C) The scatter plots show Pearson's correlation of LCK expression with expression of CD247. (D) The scatter plots show Pearson's correlation of LCK expression with expression of IL2RG. (E) The scatter plots show Pearson's correlation of GPX-8 expression with the expression of ITK. (F) The scatter plots show Pearson's correlation of LCK expression with the expression of PDCD1. (G) The scatter plots show Pearson's correlation of LCK expression with the expression of PIK3CD. (H) The scatter plots show Pearson's correlation of LCK expression with the expression of SH2D1A. (I) The scatter plots show Pearson's correlation of LCK expression with the expression of SLA2. LCK, lymphocyte-specific protein tyrosine kinase; BC, breast cancer. LCK, lymphocyte-specific protein tyrosine kinase; CD5, T-cell surface glycoprotein CD5; CD96, CD96 antigen; CD247, T-cell surface glycoprotein CD3 zeta chain; IL2RG, interleukin 2 receptor subunit gamma; ITK, tyrosine-protein kinase ITK; programmed cell death protein 1; PIK3CD, phosphoinositide-3-kinase; SH2D1A, SH2 domain-containing protein 1A; SLA2, Src-like adapter protein-2.

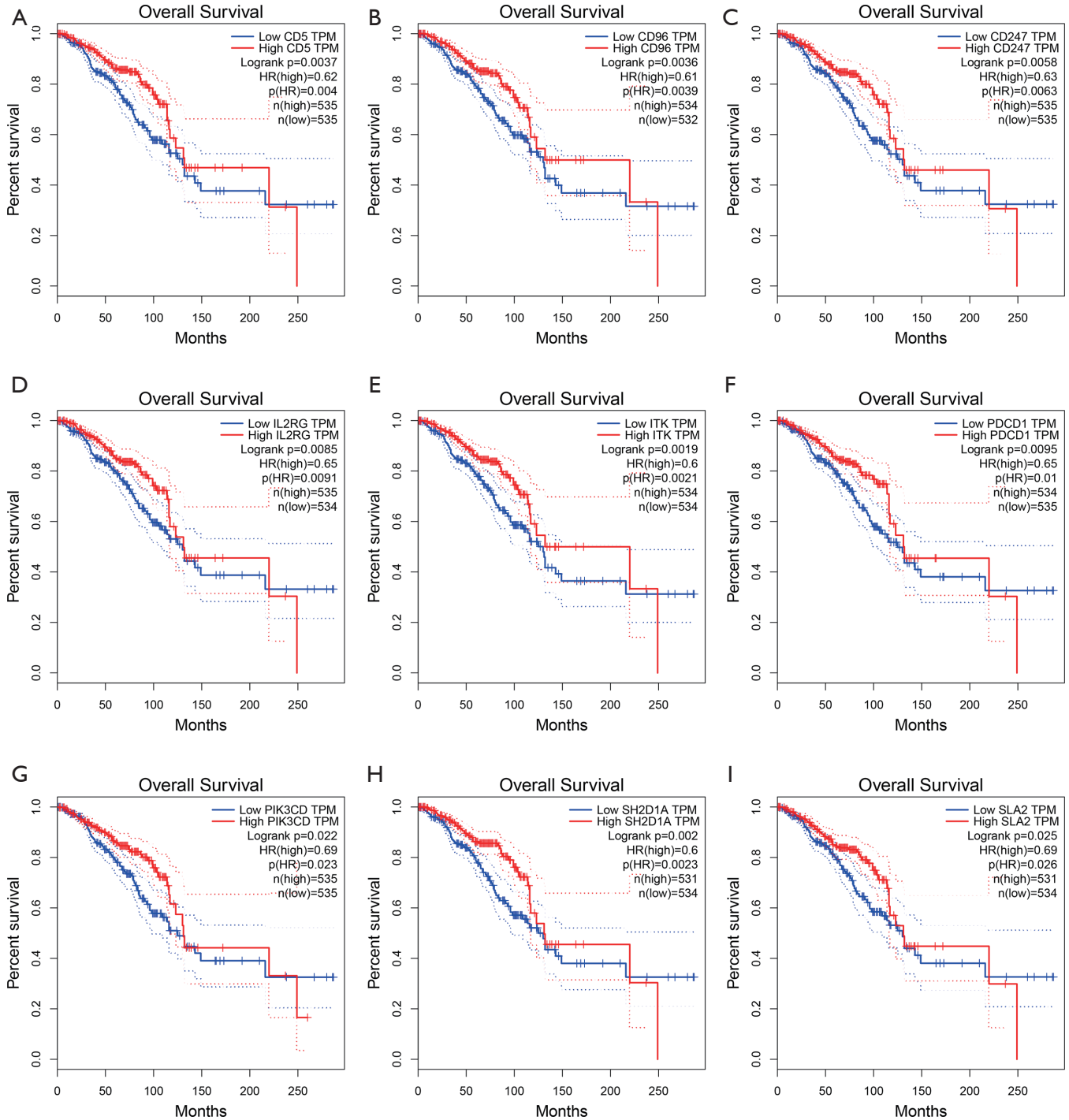


Figure 5 Prognostic analysis of genes correlated with LCK in patients with breast cancer (GEPIA). (A) The overall survival curves of CD5. (B) The overall survival curves of CD96. (C) The overall survival curves of CD247. (D) The overall survival curves of IL2RG. (E) The overall survival curves of ITK. (F) The overall survival curves of PDCD1. (G) The overall survival curves of PIK3CD. (H) The overall survival curves of SH2D1A. (I) The overall survival curves of SLA2. LCK, lymphocyte-specific protein tyrosine kinase; CD5, T-cell surface glycoprotein CD5; CD96, CD96 antigen; CD247, T-cell surface glycoprotein CD3 zeta chain; IL2RG, interleukin 2 receptor subunit gamma; ITK, tyrosine-protein kinase ITK; programmed cell death protein 1; PIK3CD, phosphoinositide-3-kinase; SH2D1A, SH2 domain-containing protein 1A; SLA2, Src-like adapter protein-2.

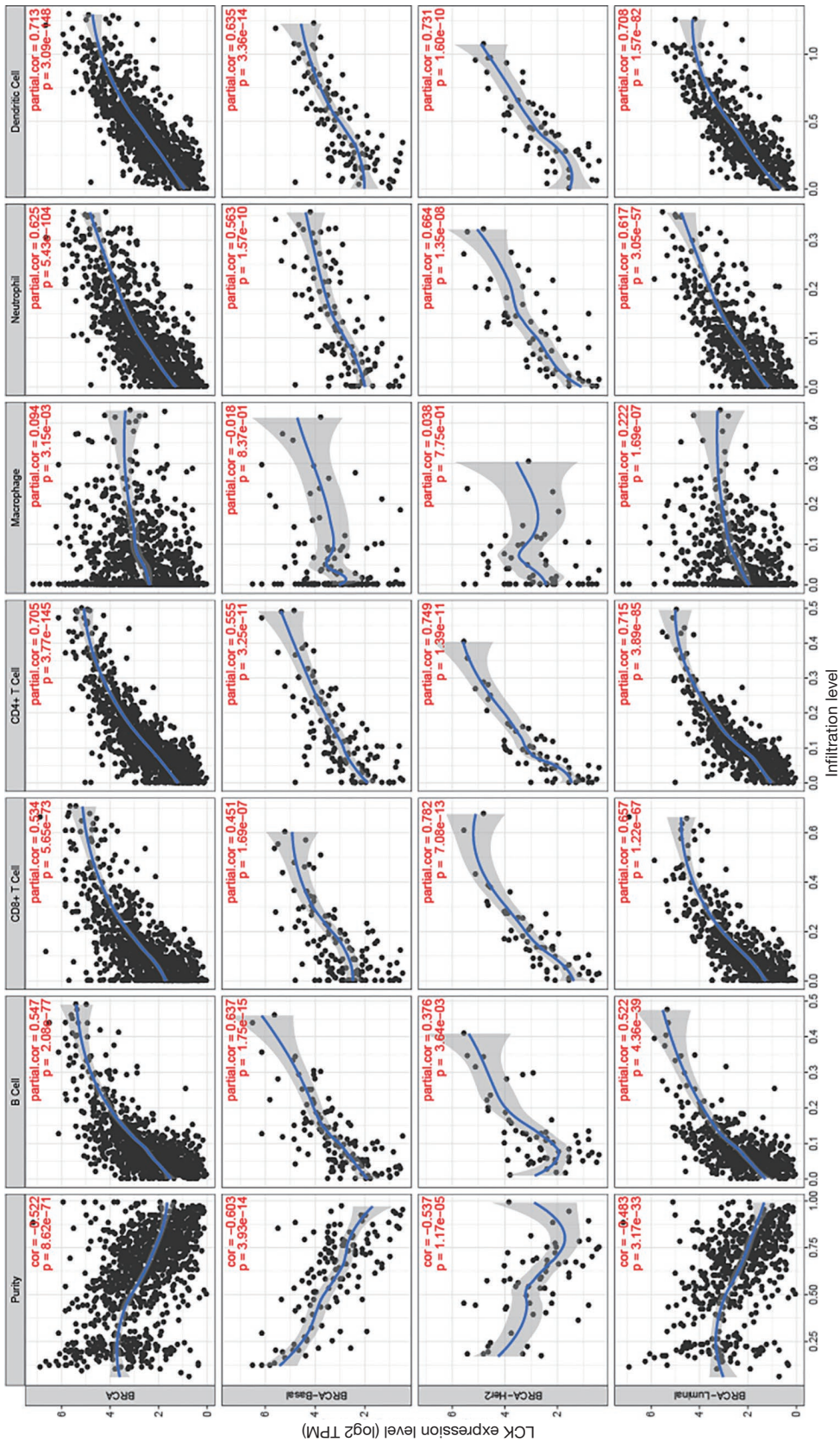


Figure 6 The relationship between LCK and immune infiltrates. LCK, lymphocyte-specific protein tyrosine kinase.

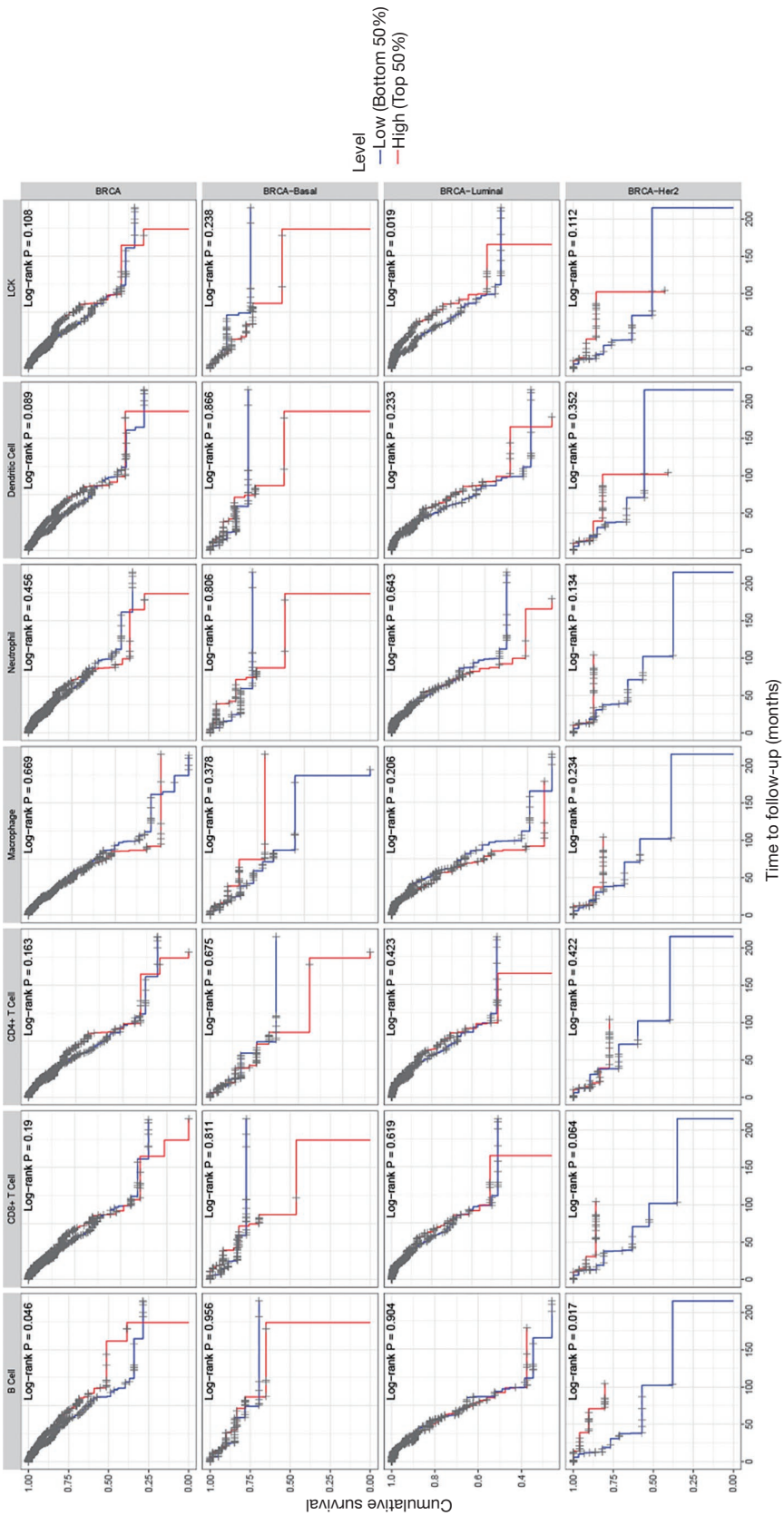
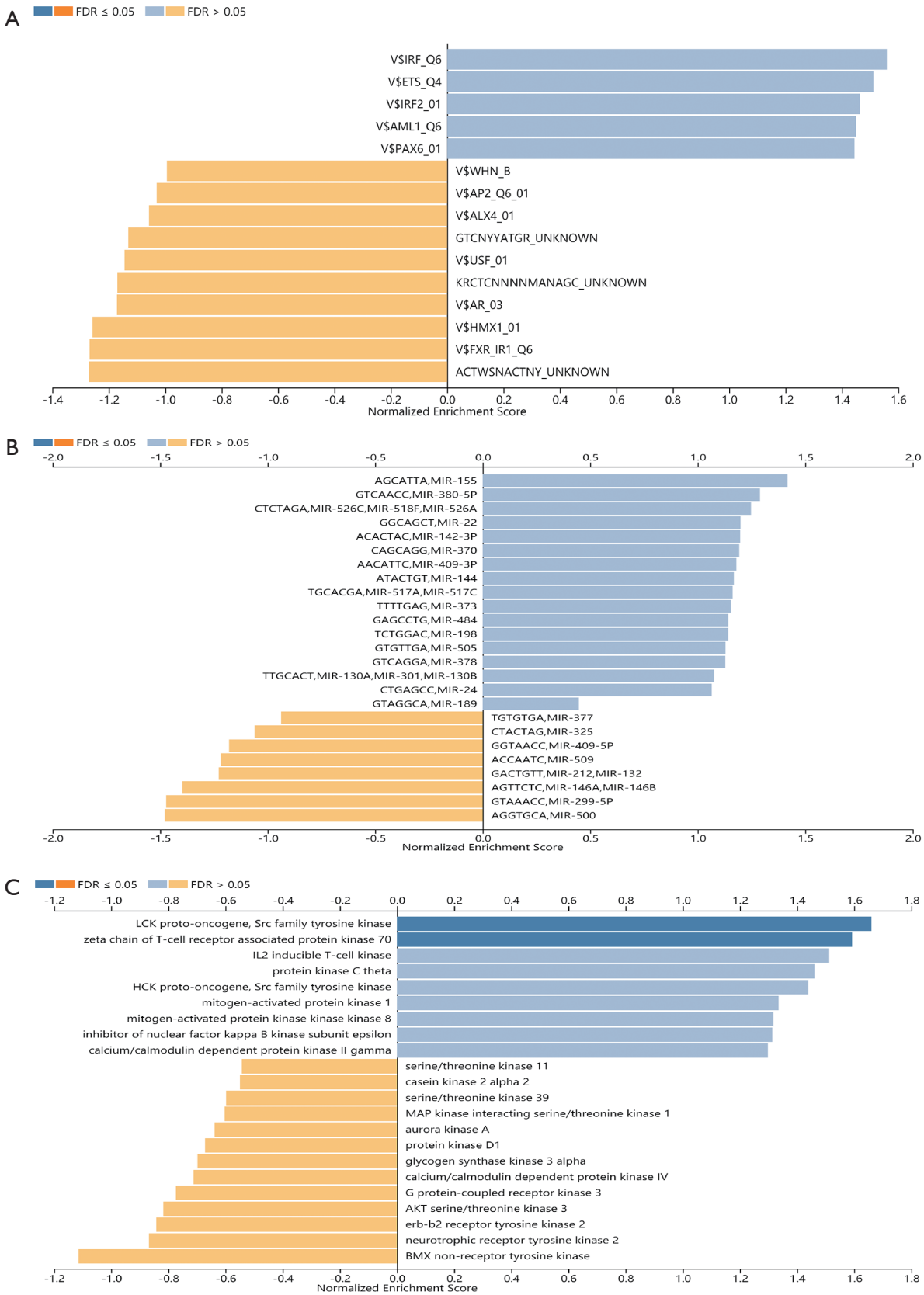


Figure 7 The relationship between immune cell infiltration and the prognosis of breast cancer.



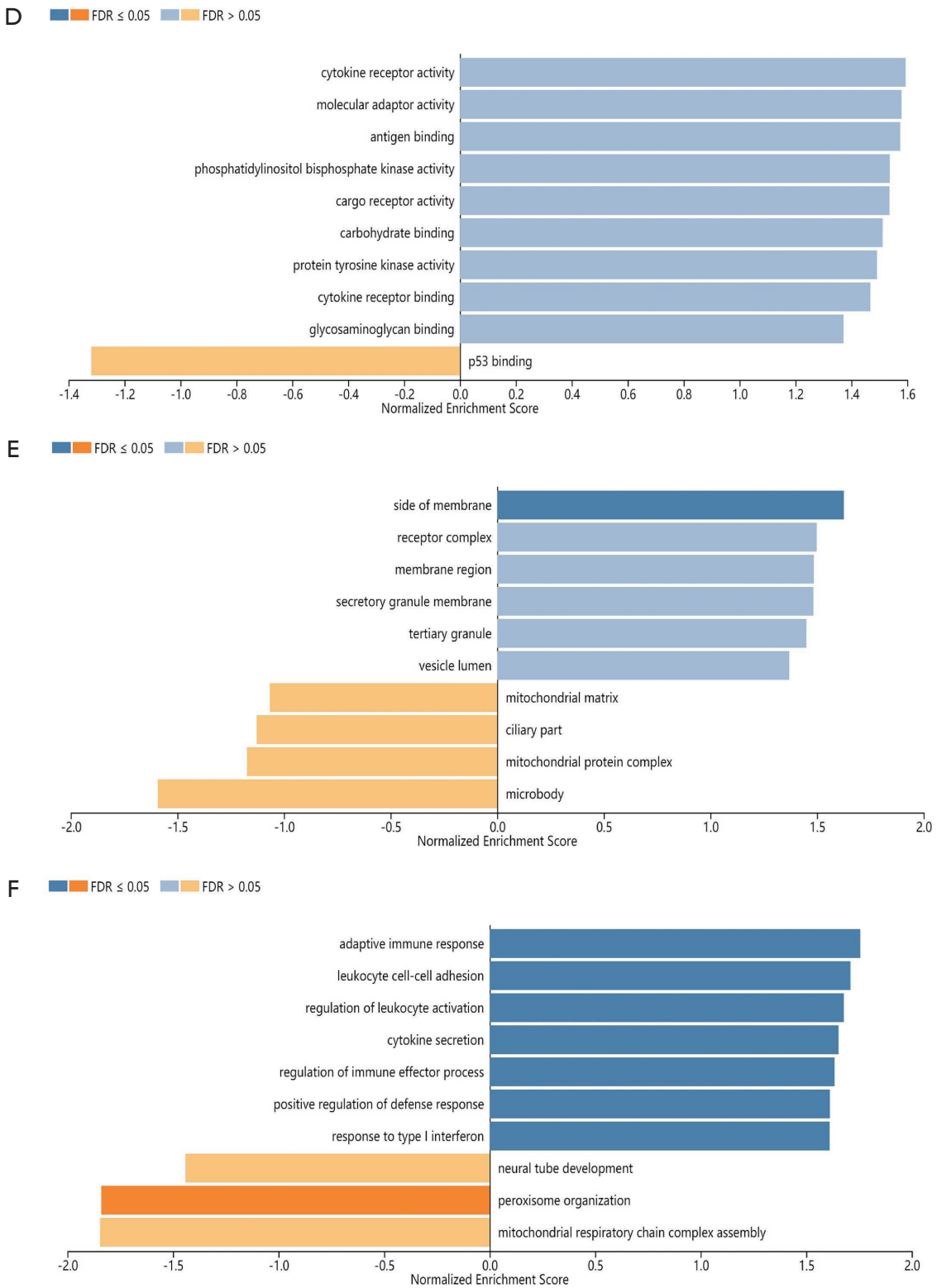


Figure 8 Functional enrichment analysis of LCK. (A) Enriched biological processes for LCK, (B) enriched cellular components for LCK, (C) enriched molecular functions for LCK, (D) kinase targets of LCK, (E) miRNA targets of LCK, and (F) transcription factor targets of LCK. LCK, lymphocyte-specific protein tyrosine kinase.

Table 1 Kinase targets of differentially expressed glutathione peroxidases in LCK (LinkedOmics)

Gene set	Description	Size	Leading edge number	P value	FDR
Kinase_LCK	LCK proto-oncogene, Src family tyrosine kinase	20	13	0.000	0.012
Kinase_ITK	IL2-inducible T-cell kinase	6	5	0.006	0.126
Kinase_MAPK3	Mitogen-activated protein kinase 3	67	17	0.026	0.588
Kinase_HCK	HCK proto-oncogene, Src family tyrosine kinase	9	5	0.028	0.308
Kinase_PRKCQ	Protein kinase C theta	13	6	0.036	0.296

LCK, lymphocyte-specific protein tyrosine kinase.

Table 2 miRNA targets of differentially expressed glutathione peroxidases in BC (LinkedOmics)

Gene set	Size	Leading edge number	P value	FDR
AGTCAGC, miR-345	22	6	0	0.11743
CTTTGTA, miR-524	117	31	0	0.15266
CAGCCTC, miR-485-5P	47	11	0	0.36502
TTTGCAG, miR-518A-2	66	12	0	0.53366
AGGTGCA, miR-500	27	9	0.017857	0.23486
AGCATTA, miR-155	46	13	0.031579	1

BC, breast cancer; Leading edge num, the number of leading edge genes.

155 as the 6 most significant miRNA targets of *LCK* in BC. Also, a number of key TFs were found to have a regulatory association with the differential expression of *LCK* in BC (*Figure 9*). Of note, AP1, SRF, and E2F1 were indicated to be key targets in the V$IRF Q6, RYTTCCTG V$ETS2B, and V$RFX1 01 TF-target networks. Based on these results, it could be seen that there were many potentially significant regulators of LCK in BC.

HPA analysis

HPA was used to analyze the differences in the levels of LCK protein expression between normal and BC tissues. The results showed that LCK protein was overexpressed in BC as compared to normal tissue. An analysis of the significantly correlated genes for protein expression was also performed, and the results are shown in *Figure 10*.

Discussion

With the development of advanced diagnostic and therapeutic methods, the lifespan of patients with BC has been lengthened; yet, BC is still the biggest contributor to cancer-related deaths in women. Mechanistically, tumor formation, drug resistance, and immune response in BC are still poorly understood, which results in the poor management of patients with BC. Therefore, more sensitive and specific novel biomarkers for the early diagnosis of BC are needed, as are novel therapeutic targets. It has been well established that aberrations in genes are important factors contributing to tumorigenesis, drug resistance, and tumor immunity. Various gene mutations have been reported in BC, including *LCK* mutation, which has also been found in other cancers. However, the fundamental biological functions of LCK in BC are still unexplored. In the current research, we comprehensively analyzed the expression levels and prognostic value of *LCK* in BC.

We found that LCK was significantly overexpressed in BC tissues compared to normal samples, and that overexpression of LCK is associated with an adverse prognosis in patients with BC. The expression of LCK may be influenced by age, histological subtype, co-mutation status, cancer status, and nodal metastasis. To date, few studies have reported on the expression of LCK in BC (9,15,24), and the relationship between LCK expression levels and prognosis remains to be explored. Bai *et al.* revealed that LCK had a significant

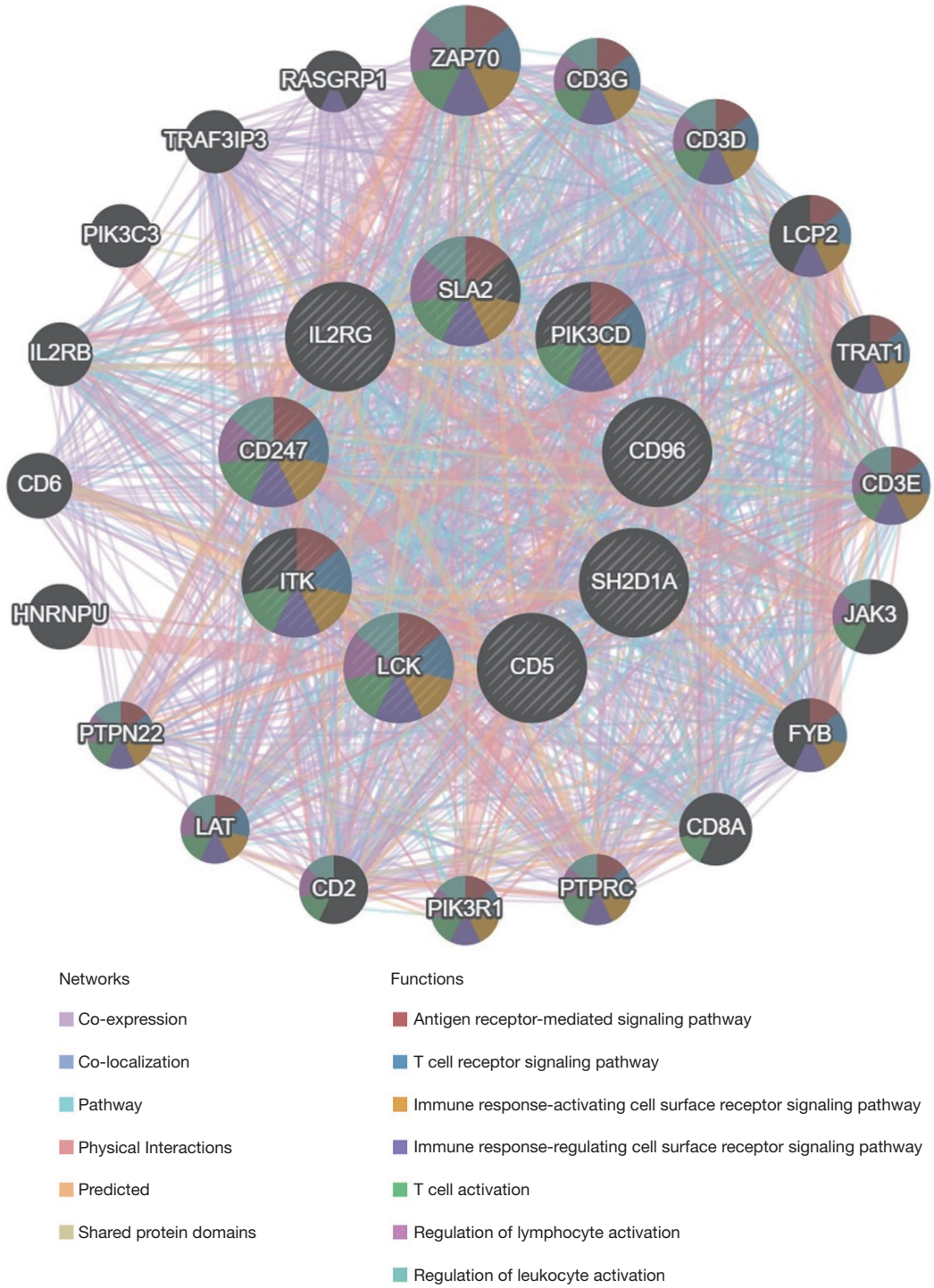


Figure 9 Protein-protein network of LCK and its significantly associated genes. LCK, lymphocyte-specific protein tyrosine kinase.

relationship with immune infiltration (15). Another study reported that LCK expression can act as a potential biomarker for predicting the recurrence of colon cancer (10). Additionally, LCK expression has also been detected in lung cancer (24). There are several correlated significant genes of LCK that including CD5, CD96, CD247, IL2RG,

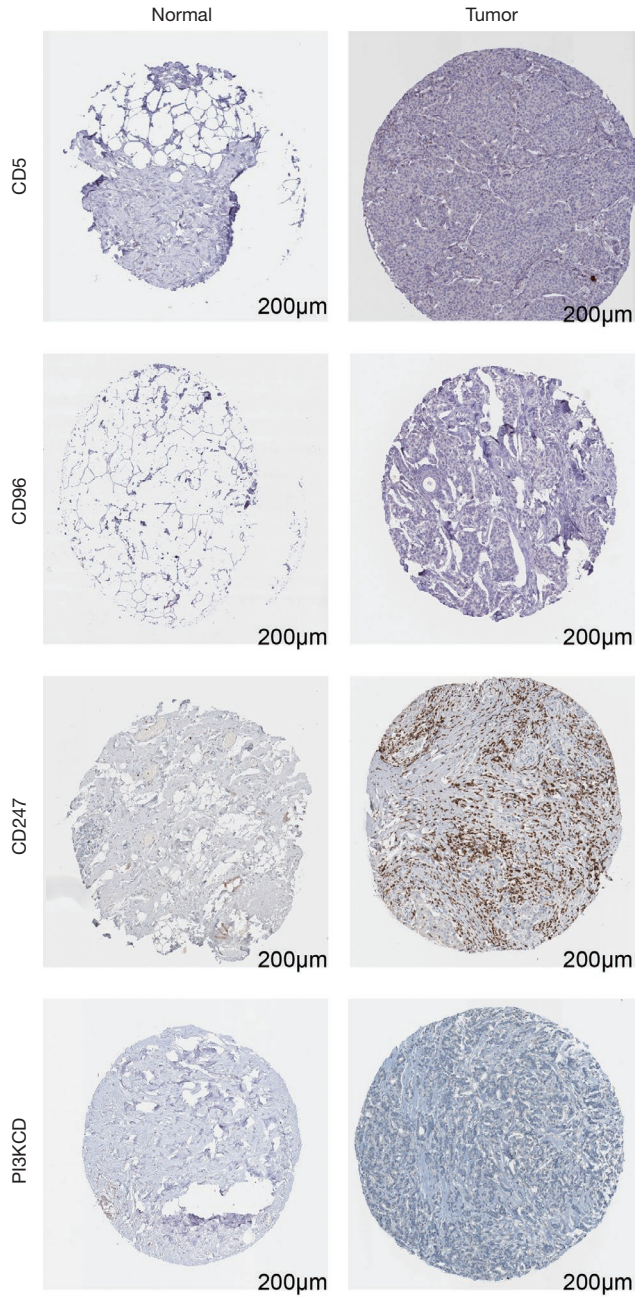


Figure 10 LCK protein expression levels in samples of breast cancer tissue and normal breast tissue (HPA). LCK, lymphocyte-specific protein tyrosine kinase; HPA, Human Protein Atlas.

ITK, PDCD1, PIK3CD, SH2D1A, and SLA2. CD5 has been observed to play a role in multiple types of lymphoma (25,26), and it may act as a regulator of cancer immunity (27). Also, CD96 was detectable in hepatocellular carcinoma, and was shown to be involved in the prognosis of patients with the disease through its regulation of the fate of natural killer cells (28). Overexpression of CD96 has been indicated to be a positive biomarker of colorectal cancer (29). In one study on ovarian cancer, downregulation of CD247 was observed, and the gene was also found to affect patient prognosis through immune cell regulation (30). ITK has been shown to be a biomarker of malignant T-cell lymphoma, and its inhibition can induce tumor cell death via TCR signaling pathway inhibition (31). Another study showed that PIK3CD promoted the growth and invasiveness of colorectal cancer cells through AKT/GSK-3β/β-catenin signaling activation (32). Moreover, PDCD1 also plays a role in immune regulation (33). However, no studies have been carried out to investigate the relationship between SLA2 and SH2D1A and cancer. All of this evidence hints that LCK and its associated genes play important roles in the pathogenesis of various cancers.

Despites of LCK was a regulator of T-cell receptor (TCR) signaling, the relationship between LCK and the tumor infiltrating lymphocytes (TIL) of BC did not have been explored. To investigate the biological functions of LCK, we performed a functional enrichment analysis of LCK in BC, and the results showed that LCK plays roles in immune regulation, including in lymphocyte-mediated immunity, activation of natural killer cells, regulation of leukocyte activation, and regulation of viral defense. The tumor microenvironment plays a crucial role in cancer progression and therapeutic efficacy, and immune infiltrates are the major constituents of the microenvironment. In our study, we investigated the relationship between LCK and immune infiltration in multiple BC subtypes. We found infiltration by B cells, CD8+ T cells, CD4+ T cells, neutrophils, and dendritic cells to have a positive correlation with LCK, but LCK was negatively correlated with tumor purity. A previous study showed that LCK had a significant impact on immune infiltration in BC, and that the overexpression of LCK was associated with a good prognosis (15). Thakur *et al.* revealed that patients with BC who had high levels of activated T cells had a longer time to progression than those who died not (34). Our current research revealed the following association of patient OS with immune infiltration in BC: the higher the level of tumor-infiltrating B-cells, the better the prognostic outcome. The study also highlighted the roles of other immune cells in BC.

Gene expression is regulated by various factors, including microRNAs and kinases. In our study, we found that there were several kinases [mitogen-activated protein kinase 3 (MAPK), HCK proto-oncogene, and protein kinase C

(PKC) theta] and microRNAs (miR-345, miR-524, miR-485-5P, miR-518A-2, miR-500, and miR-155) associated with the expression of LCK in BC. MAPK 3 is reported to be a promoter of tumorigenesis in AML. Numerous cellular functions, such as cell proliferation, differentiation, migration, and apoptosis, are regulated by MAPK pathways. Thus, aberrations of MAPK signaling pathway components play a central part in cancer development and progression (35), and aberrant MAPK3 activation has been observed in colorectal cancer (36). Also, in gastric cancer, overexpression of MAPK3 is associated with cisplatin resistance (37). Hematopoietic cell kinase (HCK), part of the cytoplasmic tyrosine kinase SRC family, can enhance the proliferation and survival of cells through physically associating with oncogenic fusion proteins and functionally interacting with receptor tyrosine kinases. Activation of HCK leads to colorectal cancer progression, and HCK is also observed in multiple myeloma and acute lymphoblastic leukemia (35,38). The PKC family is divided into distinct protein classes with various cellular functions. When activated, PRKCQ can promote the growth ability of TNBC. Among the miRNA targets of LCK, miR-345-5p, miR-524, miR-485-5p, and miR-518 act as tumor suppressors in certain cancers (39-42), and the overexpression of miR-155 was found to indicate drug resistance (43,44). Various TFs associated with LCK have also been found in other cancer types (45,46). The observations of the studies described above suggest an important role of LCK and its associated miRNAs and kinases in multiple cancers. In the present study, HPA analysis demonstrated that LCK and its correlated genes showed elevated expressions in BC samples as compared to normal breast samples. However, the limitation of this study is that absence of validation experiments.

Conclusions

In the present work, we performed a collective analysis of the expression and prognostic significance of LCK in BC, and investigated the biological events related to the progression of BC. The findings indicate that LCK is differentially expressed in BC and that its overexpression is linked to a poor survival outcome. Moreover, the results we obtained suggest that tumor immune status is an important factor for the prognostic outcomes of patients with BC, and that it could potentially serve as an indicator of prognosis in such patients. Regulatory network analysis of LCK showed its differential expression in BC to be implicated in immune cell regulation and activation, including lymphocytes, natural killer cells, and leukocytes, through a variety of tumor-related kinases (ITK and MAPK3), miRNAs (miR-345 and miR-524) and TFs (AP1, SRF, and E2F1), regulating cell proliferation, cell cycle progression, apoptosis, and survival. The limitations of the study include the small number of clinical samples and analysis using miRNA correlated genes. In future, functional validation should be performed to confirm the results obtained.

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Footnote

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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. This study was approved by the Academic Committee of Guangdong Medical University and conducted according to the principles of the Helsinki Declaration (as revised in 2013).

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