Article

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Long Noncoding RNA LINC01614 is a Diagnostic and Prognostic Marker for Breast Cancer

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Background: The long intergenic non-coding RNA 01614 (LINC01614) is aberrantly expressed in various malignancies, suggesting its role in oncogenesis. However, it has not been well studied in breast cancer.
Methods: The cancer genome atlas databases (TCGA) and public database of breast cancer gene-expression miner (bcGenExMiner) were utilized to analyze the prognostic role of LINC01614 in breast cancer. Kaplan–Meier, and Cox regression analyses were conducted for survival analysis. Nomograms were built to predict survival. We used deconvolution-based methods, such as TIMER (Tumor Immune Estimation Resource) and CIBERSORT (cell-type identification by estimating relative subsets of RNA transcripts), to explore the relationship between LINC01614 and immune cell characteristics.
Results: The very abnormal expression of LINC01614 was found in 14 types of malignancy, including breast cancer. The LINC01614 was significantly overexpressed in human epidermal growth factor receptor 2 (HER2)+, estrogen receptor (ER)+, progesterone receptor (PR)+, and non-triple negative breast cancer (non-TNBC). According to survival analysis, the higher expression of LINC01614 was related with poor survival. The co-expressed genes analysis exhibited that LINC01614 was closely associated with the collagen-associated process and phosphoinositide 3-kinases-protein kinase B (PI3K-Akt) signaling pathway. Moreover, this study has explored the association among LINC01614 expression, tumor-infiltrating immune cells, and the efficacy of chemotherapeutics.
Conclusions: Our data reveal the expression pattern of LINC01614 in breast carcinoma with different molecular subtypes. The results also indicated that the LINC01614 could be a novel diagnostic and prognostic marker for breast carcinoma.

Keywords: lncRNA; LINC01614; breast cancer; prognostic marker; diagnostic marker

Introduction

Breast cancer (BC) is the most commonly diagnosed carcinoma in females, accounting for the 2nd leading cause of cancer-related death (after lung carcinoma), with approximately 43,600 estimated deaths in the USA in 2021 [1]. According to the available epidemiological data, an increasing incidence of breast cancer is observed due to advanced diagnostic technology and the continuous extension of life expectancy [2].

Tumor biomarkers have been extensively studied for their ability to diagnose or predict prognosis with excellent specificity and sensitivity [3]. Recent evidence shows that long non-coding RNA (lncRNA) is related closely with the progression and occurrence of multiple human carcinomas, including breast cancer [4–6]. Moreover, accumulating reports have also shown that lncRNA can serve as promising diagnostic and prognostic biomarkers in multiple malignancies [7], such as colorectal cancer [8], pancreatic cancer [9], gastric cancer [10], cervical cancer [11], and so on. Hence, it is crucial to explore potent lncRNA related with the diagnosis as well as prognosis of BC.

LINC01614, a long intergenic non-protein coding RNA with 2180 nucleotides, is encoded on chromosome 2q35. It is reported that LINC01614 was abnormally expressed in several types of carcinomas and was closely associated with malignant biological behavior and poor clinical outcomes [12–14]. For instance, a previously published study has shown that LINC01614 promotes pancreatic cancer progression through hyperactivating wnt/β-catenin signaling [15]. Also, LINC01614 stimulated the development of lung cancer via regulating forkhead box pro-

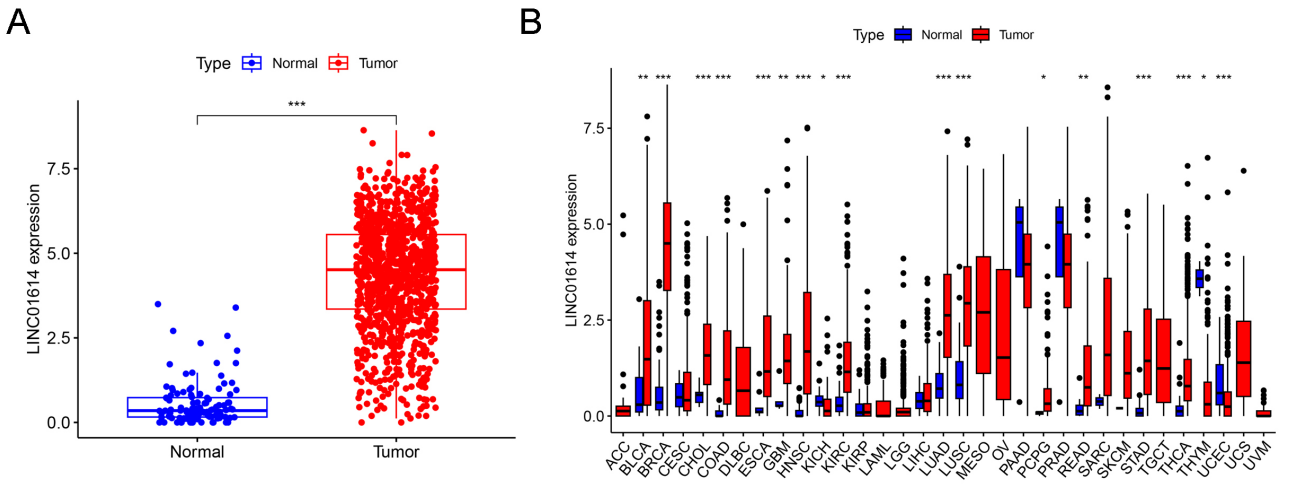

Fig. 1. LINC01614 expression levels in breast tumors and different types of tumors. (A) LINC01614 expression in cancer was dramatically higher than normal control. (B) The comparison of LINC01614 expression between different kinds of tumors and normal control. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

tein P1 (FOXP1) [16]. Nevertheless, the specific role of LINC01614 in BC is still not elucidated.

Here, LINC01614 was overexpressed in BC and was related closely to poor clinical outcomes. We also explored the association between LINC01614 and tumor-infiltrating immune cells, as well as the efficacy of chemotherapeutics. Our findings hint that LINC01614 may serve as a novel diagnostic and prognostic marker in breast cancer.

Material and Methods

Data Collection

We extracted the RNA sequencing data as well as associated clinical characteristics of breast cancer samples from the cancer genome atlas (TCGA) database (https://portal.gdc.cancer.gov/). We converted the fragment per kilobyte per million (FPKM) format of the RNA sequencing data to transcript per million reads (TPM). Then RNA sequencing data was analyzed based on the corresponding clinical characteristics.

Correlation between LINC01614 Expression and Breast Cancer

We compared the expression of LINC01614 in BC and normal controls. In addition, the LINC01614 expression level was also investigated in various cancers obtained from TCGA. The Kaplan–Meier method explored the association between LINC01614 expression and survival in the TCGA and Gene Expression Omnibus (GEO) validation datasets. The bc-GenExMiner v4.8 (breast cancer gene-expression miner v4.8), which included multiple GEO breast cancer datasets, validated our result. For the overall survival (OS) analysis, the portal contained 818 patients and was integrated from five GEO databases, including GSE1456, GSE20685, GSE42568, GSE43358, and GSE12276. For the disease free survival (DFS) analysis, the portal contained 2452 patients and was integrated from five GEO databases, including GSE1456, GSE4922, GSE6532, GSE9195, GSE16391, GSE1961, GSE19615, GSE17907, GSE20711, GSE20685, GSE21653, E_MTAB_365, GSE42568, GSE43358, GSE12276. The log-rank test detected the significance of the survival difference. Nomograms were then developed to predict OS as well as progression-free survival (PFS) using R package "survival" (version 3.2-10, R Foundation for Statistical Computing, Vienna, Austria) and "rms" (version 6.2-0, R Foundation for Statistical Computing, Vienna, Austria). We estimated the consistency between the observed outcomes and the predictions based on the nomogram via the plotting of calibration curves. R (version 4.2.2, R Foundation for Statistical Computing, Vienna, Austria) was utilized for statistical analysis.

The Identification of DEGs and Pathway Analysis

Those with Pearson correlation coefficients >0.7 and p-value < 0.001 were regarded as LINC01614-associated differentially expressed genes (DEGs). Then, the Kyoto encyclopedia of genes and genomes (KEGG) and gene ontology (GO) enrichment analyses of co-expressed genes were conducted with the utilization of the R package "cluster Profiler" (version 3.14.3, R Foundation for Statistical Computing, Vienna, Austria). GO analysis consists of three parts, including molecular function (MF), biological processes (BP), and cell composition (CC). The gene set enrichment analysis (GSEA) was performed by utilizing the R package "org.Hs.eg.db".

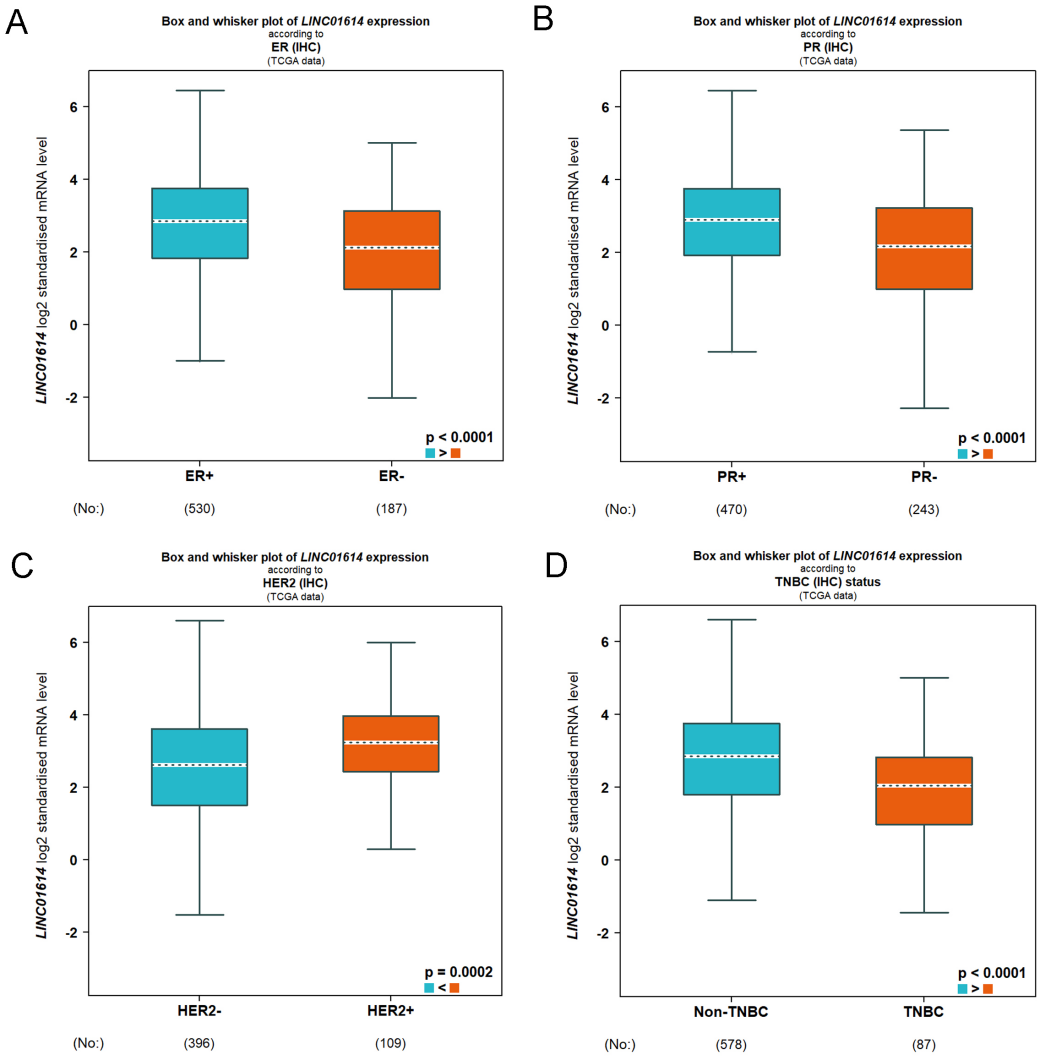

Fig. 2. LINC01614 expression levels in different subtypes. Expression of LINC01614 in different subtypes stratified by ER (A), PR (B), and HER2 (C). (D) The expression of LINC01614 in TNBC and non-TNBC.

Investigation of Tumor-Infiltrating Immune Cells

Expression pattern of LINC01614 related with immune cell characteristics was determined by TIMER (Tumor Immune Estimation Resource) and CIBERSORT (Cell-type Identification by Estimating Relative Subsets of RNA Transcripts) methods, which evaluate the immune infiltration status among breast cancer samples from the TCGA project. The Spearman correlation analysis explored association between LINC01614 expression and the immune infiltrated cells. A lollipop diagram illustrates the correlation coefficients. This analysis was executed by the R package "ggplot2".

Association between LINC01614 and Clinical Treatment

The half inhibitory concentration (IC50) of common chemotherapeutic drugs in the breast invasive carcinoma (BRCA) dataset from the TCGA project was calculated. Difference of IC50 between subgroups stratified by the LINC01614 expression was compared with the Wilcoxon signed-rank test. Results were represented as box drawings using the R package "ggplot2".

Results

LINC01614 Expression Profiles in Pan-Cancer Datasets

TCGA data suggested that LINC01614 expression was upregulated in breast cancer tissues compared with normal control ($p < 0.001$, Fig. 1A). Moreover, the pan-cancer analysis also indicated that LINC01614 was overexpressed in 14 out of 33 cancer types, such as colon adenocarcinoma (COAD), head and neck squamous cell carcinoma (HNSC) and bladder urothelial carcinoma (BLCA) (Fig. 1B). Thus, a significant role may be played by LINC01614 in the development of breast cancer.

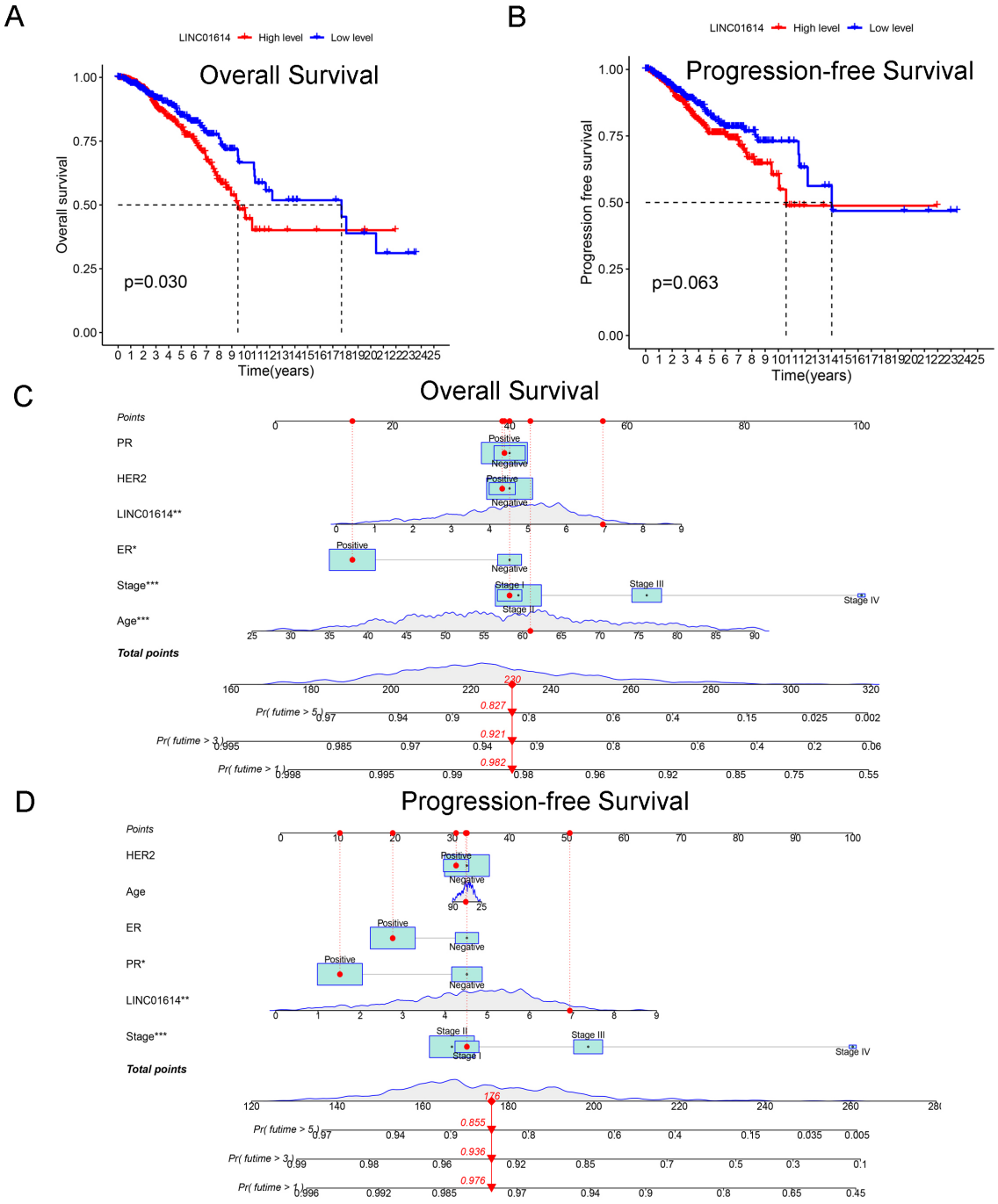

Fig. 3. The prognostic value of LINC01614 expression in breast cancer patients. (A) Association between LINC01614 expression and OS. (B) Association between LINC01614 expression and PFS. (C) Nomograms constructed for OS. (D) Nomograms constructed for PFS. Variables with an asterisk were proved to be an independent prognostic factors. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

LINC01614 Expression Pattern in Distinct Subtypes

The expression of LINC01614 in different molecular subtypes was analyzed using bc-GenExMiner v4.8, a breast cancer-associated web portal based on published transcriptomic and clinicopathological data. Fig. 2A–C showed that LINC01614 was significantly overexpressed in human epidermal growth factor receptor 2 (HER2)+, estrogen receptor (ER)+, progesterone receptor (PR)+ patients compared with those with ER– PR–, or HER2–. Moreover, Fig. 2D showed that LINC01614 was significantly overexpressed in non-triple negative breast cancer (non-TNBC) patients compared with TNBC.

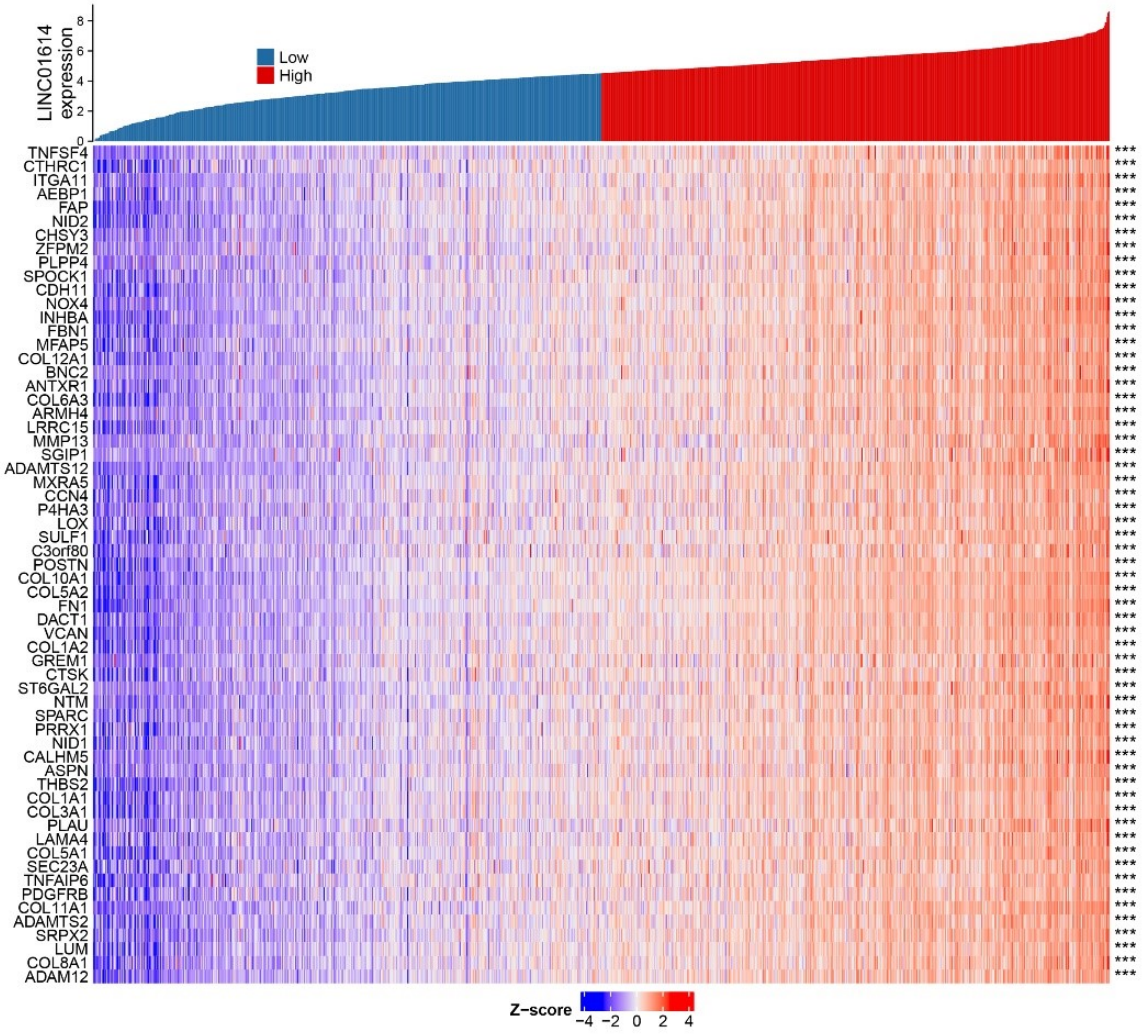

Fig. 4. Heatmap of the top 61 DEGs significantly correlated with LINC01614 expression in breast cancer. $^{***}p < 0.001$.

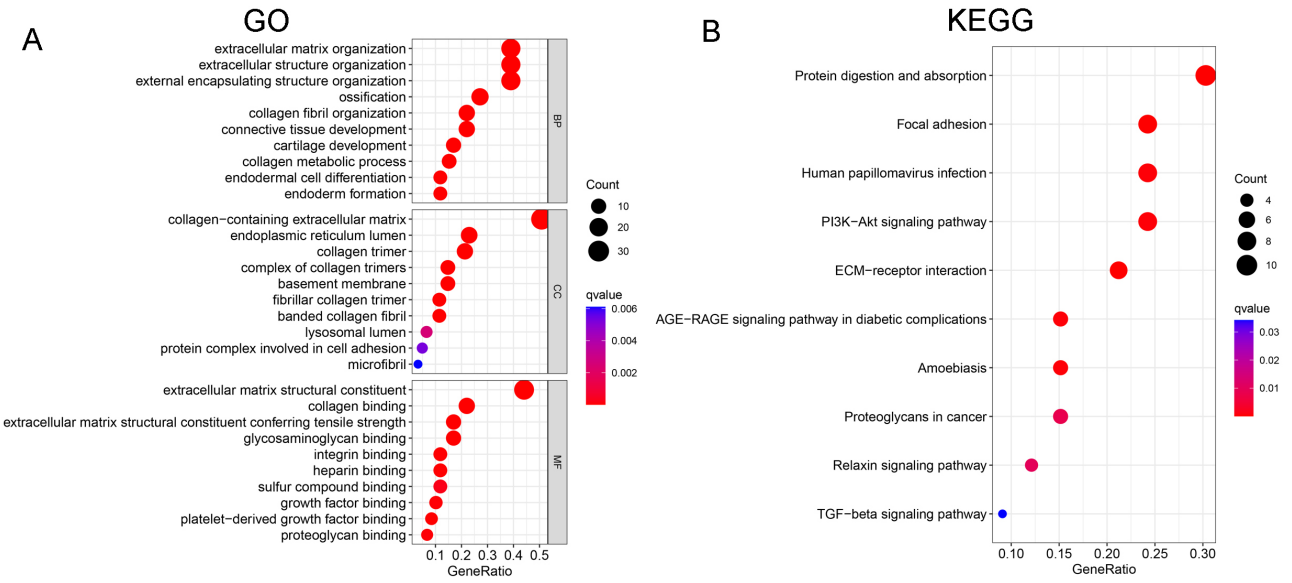

Fig. 5. Pathway analyses of LINC01614-associated DEGs in breast cancer. (A) GO pathway analysis of DEGs. (B) KEGG pathway analysis of DEGs.

Prognostic Role of LINC01614 in Breast Cancer

The Kaplan–Meier analysis assesses whether LINC01614 expression could be used to predict clinical outcomes of breast cancer. Fig. 3A showed that breast cancer with upregulated expression of LINC01614 had a dramatically shorter OS (p = 0.03) than those with low expression. Regarding PFS analysis, although there is no statistical difference, patients with a relatively higher level of LINC01614 expression had a worse clinical outcome, especially within the 2–10 years of follow-up (Fig. 3B). The bc-GenExMiner v4.8 validated our result. As shown in **Supplementary Fig. 1A,B**, although there was no statistical difference, patients with relatively high LINC01614 expression had worse OS and PFS, especially after two years of follow-up, which were similar to our data. Moreover, after deleting the inconclusive data (equivocal, indeterminate, or unknown), patients with clinical data in TCGA were divided into luminal, HER2-positive, and triple-negative types based on PAM50. As shown in **Supplementary Fig. 2A–C**, a survival difference was seen in each of those subgroups.

Subsequently, prognostic nomograms were designed by including several variables (ER, PR, HER2, stage, and LINC01614 expression). A total point could be calculated for individual breast cancer patients according to their point scale in the nomogram. We could forecast the 1-, 3-, and 5-year survival clearly through this approach. For example, the clinicopathological features of randomly selected patients resulted in a total point score of 230, resulting in the 1-, 3-, and 5-year survival of 98.2%, 92.1%, and 82.7%, respectively, for OS (Fig. 3C). Meanwhile, Fig. 3D illustrated that a total point score of 176 represented the 1-, 3-, and 5-year survival of 97.6%, 93.6%, and 85.5%, respectively, for PFS. A higher score was generally related with a poor prognosis. Moreover, a plot of the calibration curves revealed good consistency between the predicted survival probability by the nomogram and the observed survival probability (**Supplementary Fig. 3**).

Functional Annotation of LINC01614-Associated DEGs in Breast Cancer

A Pearson correlation coefficient of >0.7 and $p < 0.001$ was set to screen for co-expressed genes of LINC01614. As a result, the heatmap of the top 61 DEGs significantly correlated with the LINC01614 expression pattern (Fig. 4). Subsequently, LINC01614-associated DEGs in breast cancer were analyzed using GO and KEGG analysis. GO analysis showed that genes associated with the BP term were significantly enriched in the collagen metabolic process, extracellular matrix organization, and ossification. For the CC term, genes were significantly enriched in the collagen-containing extracellular matrix. Moreover, the genes related to collagen binding and extracellular matrix structural constituent were enriched in the MF term (Fig. 5A). As shown in Fig. 5B, human papillomavirus infection, PI3K-Akt (phosphoinositide 3-kinases-protein kinase B) signaling pathway, focal adhesion, and protein digestion and absorption were the top pathways based on the KEGG analysis. Further, GSEA analysis showed that LINC01614 was significantly enriched in ECM receptor interaction, focal adhesion, and steroid hormone biosynthesis pathway (**Supplementary Fig. 4**).

Estimation of Tumor-Infiltrating Immune Cells

Since lncRNA may be associated with immune-related genes, we investigated the association between the expression pattern of LINC01614 and the tumor immune microenvironment. As a result, the expression level of LINC01614 was positively related to tumor-infiltrating immune cells, such as macrophages, neutrophils, and CD4 memory T cells (Fig. 6A,B). Meanwhile, it was negatively associated with CD8 T cells, activated natural killer (NK) cells, T cells regulatory (Tregs), monocytes, and memory B cells. Based on the Spearman correlation analysis, Fig. 6 displayed a lollipop diagram showing the detailed results.

Analysis of the Correlation between LINC01614 and Chemotherapeutics

Based on the BRCA (breast invasive carcinoma) dataset, we attempted to explore the association between the expression pattern of LINC01614 and the efficacy of standard chemotherapeutics available in the TCGA project. Fig. 7 showed that a high expression level of LINC01614 was dramatically related to a high IC50 of chemotherapeutic agents including cyclophosphamide ($p < 0.001$), lapatinib ($p < 0.001$), palbociclib ($p < 0.001$), ribociclib ($p < 0.001$), and tamoxifen ($p < 0.001$). These data hint that LINC01614 could act as a potential predictor for chemotherapy sensitivity.

Discussion

Even though endocrine therapy and anti-HER2-targeted therapy have greatly improved breast cancer treatment, the BC patient's prognosis is still unsatisfactory. Hence, exploring the underlying molecular mechanisms and identifying potent prognostic markers and potential therapeutic targets for breast cancer is noteworthy and clinically relevant.

Researchers have found that lncRNAs are crucial in cancer pathogenesis and progression [17]. Some pivotal lncRNAs have been identified as promising diagnostic and prognostic biomarkers, such as HOTAIR [18], SNHG1 [19], and ANRIL [20]. Moreover, studies have validated the prognostic value of lncRNAs in breast cancer [21,22].

This study used the TCGA database to analyze the LINC01614 expression in 33 cancer types. LINC01614 was found to be abnormally overexpressed in 14 types of malignancy, especially in breast cancer, which indicated its oncogenic role in human carcinomas. BC is a heteroge-

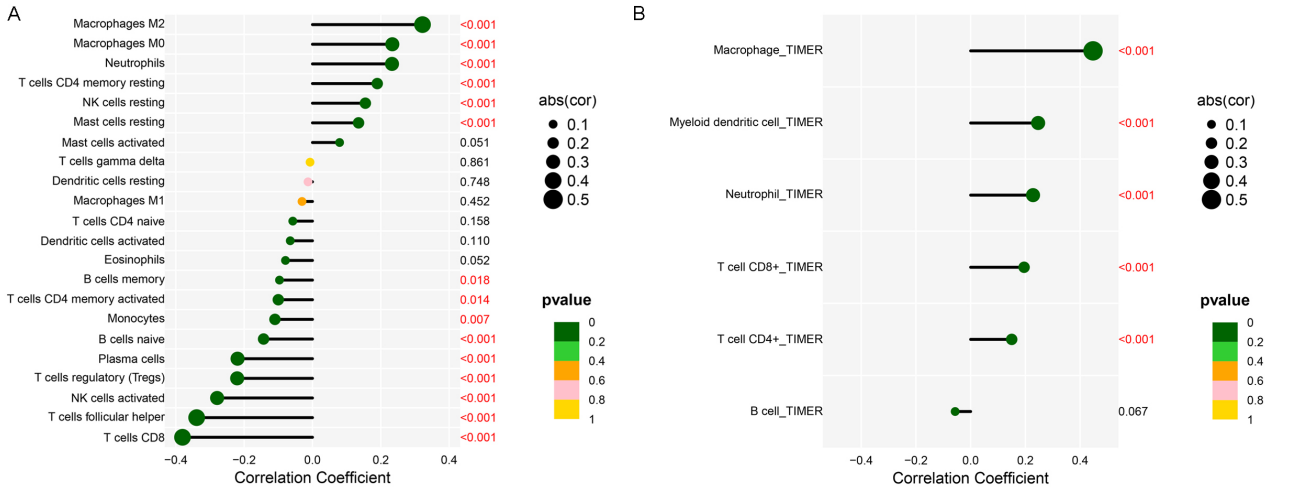

Fig. 6. A lollipop diagram showing the relationship between LINC01614 expression and the tumor immune microenvironment. (A) CIBERSORT method. (B) TIMER method.

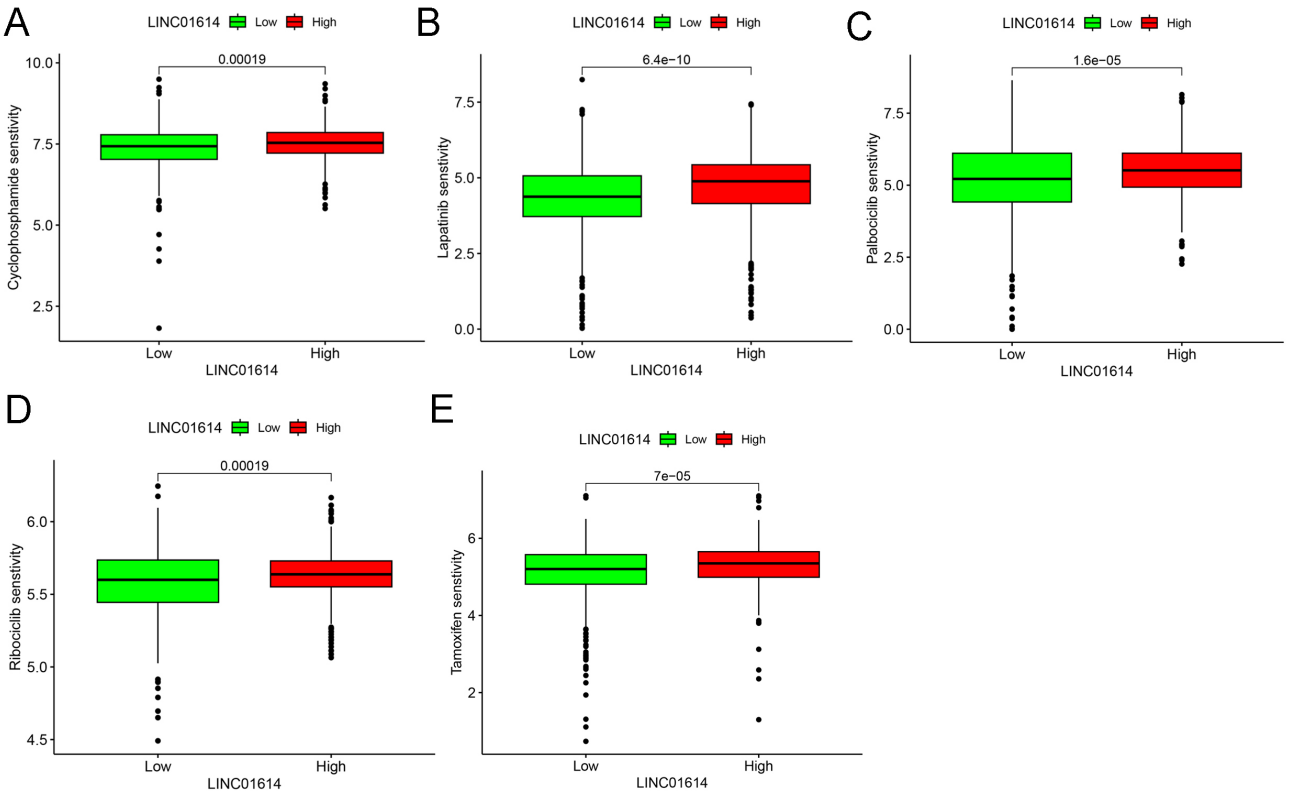

Fig. 7. Association between LINC01614 expression and sensitivity of chemotherapeutics. (A) Cyclophosphamide. (B) Lapatinib. (C) Palbociclib. (D) Ribociclib. (E) Tamoxifen.

neous disease and can be subdivided into three distinct subtypes according to three key biomarkers: Luminal group, HER-2 overexpressing group, and basal-like group, TNBC [23,24]. Consequently, we further investigated the association between LINC01614 expression and its subtypes. LINC01614 was significantly overexpressed in ER, PR, and HER-2 positive groups.

Further, the TNBC subgroup had a dramatically lower expression of LINC01614 than non-TNBC subgroups. Survival analysis showed that patients with relatively high expression of LINC01614 had poor clinical outcomes, suggesting its prognostic value in breast cancer. The PFS analysis statistic seems insignificant because of the small sample size in the TCGA project. Analysis of co-expressed

genes indicated that LINC01614 was closely related to the collagen-associated process. A previous study has shown that collagen constitutes the major component of the tumor microenvironment and is included in the process of tumor fibrosis [25]. Cancer cells could regulate transcription factors, collagen biosynthesis through mutated genes, and signaling pathways. In turn, collagen can also affect tumor cell behavior via discoidin domain receptors, integrins, tyrosine kinase receptors, and some signaling pathways. Moreover, PI3K-Akt signaling, a cancer-related pathway, was observed in the KEGG analysis [26].

Recent evidence indicated that tumor-infiltrating immune cells could affect the response to the anti-checkpoint blockade. For instance, KEYNOTE-001 showed that patients with more CD8+ T cell infiltration responded better to pembrolizumab than those with less infiltration [27]. Moreover, some studies reported that lncRNA could influence the infiltration level of tumor-infiltrating immune cells [28,29]. Therefore, a currently acknowledged method, CIBERSORT, was utilized to evaluate the association between LINC01614 expression and tumor-infiltrating immune cells [30]. Our data suggested that LINC01614 was negatively related with CD8 T cells, activated NK cells, monocytes, activated memory CD4 T cells, and memory B cells. This result suggested that high expression of LINC01614 might be associated with an immunosuppressive state in the tumor microenvironment. Additionally, we found that LINC01614 expression was associated with sensitivity to chemotherapeutics such as cyclophosphamide, lapatinib, palbociclib, ribociclib, and tamoxifen, which implied that LINC01614 could serve as a potential predictor for sensitivity chemotherapy.

Conclusions

LINC01614 aberrant expression is an important regulator of biological processes in breast cancer progression. Our study demonstrated that LINC01614 overexpression is a reliable diagnostic and prognostic marker, and the LINC01614 could be used to predict the sensitivity of some specific anti-tumor drugs.

Abbreviations

LINC01614, long intergenic non-coding RNA 01614; TCGA, the cancer genome atlas databases; bc-GenExMiner, breast cancer gene-expression miner; CIBERSORT, Cell-Type Identification by Estimating Relative Subsets of RNA Transcripts; TIMER, Tumor Immune Estimation Resource; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; TNBC, triple negative breast cancer; FOXP1, forkhead box protein P1; FPKM, fragment per kilobyte per million; TPM, transcript per million reads; GEO, Gene Expression Omnibus; OS, overall survival; PFS, progression-free survival; DEGs, differentially expressed genes; KEGG, Kyoto encyclopedia of genes and genomes; GO, gene ontology; BP, biological processes; MF, molecular function, CC, cell composition; GSEA, gene set enrichment analysis; COAD, colon adenocarcinoma; BLCA, bladder urothelial carcinoma; HNSC, head and neck squamous cell carcinoma.

Availability of Data and Materials

The data generated and/or analysed during the current study are available in the TCGA database (https://portal.gdc.cancer.gov/).

Author Contributions

WL and YQ—designed the research study; WL, YC, JC, JY, MS, MY—analyzed the data; WL and YQ—wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202335174.3.

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