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ORIGINAL RESEARCH

Prognostic Significance of Iron Metabolism Related Genes in Human Lung Adenocarcinoma

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Background: Iron metabolism related genes participate in cell proliferation, cell growth, and redox cycling in multiple cancers. Limited studies have revealed the roles and clinical significance of iron metabolism in the pathogenesis and prognosis of lung cancer.

Methods: A total of 119 iron metabolism related genes were extracted from MSigDB database and their prognostic values were determined in The Cancer Genome Atlas lung adenocarcinoma (TCGA-LUAD) dataset and the Gene Expression Profiling Interactive Analysis 2 (GEPIA 2) database. Immunohistochemistry technique and correlations with immune cell infiltration, gene mutation and drug resistance were used to identify the potential and underlying mechanisms of STEAP1 and STEAP2 as prognostic biomarkers of LUAD.

Results: The expression of STEAP1 and STEAP2 are negatively associated with the prognosis of LUAD patients both at the mRNA and protein level. The expression of STEAP1 and STEAP2 was not only negatively correlated with the trafficking degree of CD4+ T immune cells and positively related to most immune cells' trafficking degree, but also significantly associated with gene mutation status, particularly with mutations on TP53 and STK11. Four types of drug resistance showed significant correlation with the expression level of STEAP1 while 13 types of drug resistance were associated with the expression level of STEAP2.

Conclusion: Multiple iron metabolism related genes including STEAP1 and STEAP2 are significantly associated with the prognosis of LUAD patients. STEAP1 and STEAP2 might affect the prognosis of LUAD patients partially through immune cell infiltration, gene mutation and drug resistance, which indicated they were independent prognostic factors for LUAD patients.

Keywords: iron metabolism related genes, lung adenocarcinoma, prognosis, STEAP1, STEAP2

Introduction

Lung cancer is one of the main terminal cancers in patients. Lung adenocarcinoma (LUAD) is the most common type of lung cancer.[1] Although multiple novel treatment methods have been developed, the 5-year survival rate of patients with lung cancer has only improved slightly.[2,3] It remains of great clinical importance to identify novel biomarkers for prognosis prediction in LUAD patients which may help to formulate effective prevention, screening and treatment strategies. Currently, the prognosis of LUAD patients is determined by classical clinicopathological features such as histological grade, lymph node metastasis and tumor size. Unfortunately, patients with LUAD at the same stage always have different clinical results. The heterogeneity of LUAD makes the current staging system inaccurate in providing reliable prediction. Hence, it is urgent to develop new biomarkers to predict the prognosis of LUAD.

Iron, involved in multiple cellular functions, is one of the most important and abundant trace elements. Recent studies focusing on the roles of iron in cancer have shown that iron metabolism related proteins are multifunctional and may lead to malignant tumors in a way other than their functions in iron metabolism. The intense aerobic glycolysis process of tumor cells is closely related to iron metabolism.[4] Iron can, not only promote cell proliferation, but also promote

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tumorigenesis. Growing evidence has revealed that lung cancer, glioblastoma, leukemia, breast cancer, ovarian cancer, liver cancer, thyroid cancer, and colorectal cancer are closely related to the altered expression of iron metabolism related genes.[5–13] Iron metabolism related proteins have the potential to activate the STAT3-FoxM1 axis, which results in increasing generation of reactive oxygen species, triggering P53 nuclear export, promoting activation of GP130/STAT3 signaling and regulatory pathway. For example, IREB1 and IREB2 promote tumorigenesis and cancer formation.[14–17] Downregulation of FPN1 promoted myeloma cell growth in multiple myeloma.[18] At present, there is plenty of evidence that dysfunctions in iron uptake and management are the critical characteristics of lung cancer, suggesting that iron metabolism related genes and proteins have great potential to predict the progression and prognosis of lung cancer.[19–21] Further interrogation of this relationship between cancer and iron metabolism related genes may yield more mechanistic insights and opportunities for early prognostic prediction.

In this study, the prognostic values of 119 iron metabolism related genes were comprehensively investigated in lung adenocarcinoma (LUAD) patients. We found that LUAD patients with higher STEAP1 and STEAP2 tumor tissue expression levels are associated with poorer prognosis. To further elucidate the mechanisms, we performed systematical bioinformatics analyses based on the gene expression levels, immune cell trafficking degree, gene mutation status and drug resistance in LUAD patients. Overall, we demonstrated that iron metabolism related genes, especially STEAP1 and STEAP2, could be used as independent prognostic predictors in LUAD patients.

Materials and Methods

Data Acquisition and Bioinformatic Analysis

Gene expression data used in this article were obtained from TCGA-LUAD database (https://cancergenome.nih.gov/). A total number of 119 iron metabolism related genes were selected from MSigDB database (http://www.gsea-msigdb.org/gsea/msigdb). The correlation analysis and the univariate Cox regression analysis between 119 genes and the LUAD patient prognosis were determined in TCGA-LUAD dataset, which included 498 LUAD tissues and 58 adjacent normal tissues.

Survival information and gene expression data of iron metabolism related genes were verified by GEPIA2 database (http://gepia2.cancer-pku.cn/), an enhanced web server for large-scale expression profiling and interactive analysis. Kaplan-Meier survival plots were used to show the association between mRNA expression level and overall survival (OS). Hazard ratio with 95% confidence intervals (CI) between the groups were calculated using univariate Cox regression analysis.

Protein-Protein Interaction Network and Correlation Analysis Between 20 Genes

The protein-protein interaction (PPI) network of iron metabolism proteins was analyzed by the STRING database (https://cn.string-db.org/) and the GeneMANIA database (http://genemania.org/), databases of predicted functional associations between proteins, to explore the iron metabolism related hub genes in LUAD prognosis. We constructed an interaction network among candidate iron metabolism proteins, whose expression was significantly associated with the prognosis of LUAD patients both in TCGA and GEPIA 2 database. Correlation between the gene expression level of the 20 genes was calculated using Pearson correlation coefficient (r) and visualized using the heatmap generated by R package corrplot (v0.92).

Ethical Statement

All experiments were performed in accordance with relevant guidelines and regulations. All study participants provided informed consent, and this study design was approved by the ethics committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine [Agreement Number: (2020) (013)-1]. This study was performed in accordance with the Declaration of Helsinki.

Tumor Tissue Samples

Lung tissue samples were collected during surgical resection and archived under protocols that were approved by Ruijin Hospital, Shanghai Jiao Tong University School of Medicine after obtaining informed consent from the patients. All of

the samples were diagnosed as LUAD based on the tumor, lymph node, and metastasis (TNM) staging system by senior pathologist and no treatment was performed before tissue samples were collected. The basic information of patients such as smoking status, common comorbidities were collected from medical record system of Ruijin hospital.

Immunohistochemistry (IHC) Validation in LUAD

The 4 μm sections were cut from each tissue block, and they were placed in a 90°C incubator for 1 hour. After being deparaffinized in xylene and rehydrated, the samples passed through graded alcohol solutions. In a steam pressure cooker, tissue sections were placed in citrate buffer to react at 125°C for 3 minutes to perform antigen retrieval. After natural cooling, 3% hydrogen peroxide solution was used to block endogenous peroxidase for 30 minutes. After three washes with PBS solution, the sections were incubated with 3% BSA for 1 hour and then the sections were incubated with rabbit anti-STEAP1 antibody (1:250 dilution, Catlog No: #24565, Signalway Antibody, Pearland, TX, USA) and rabbit anti-STEAP2 antibody (1:100 dilution, Catlog No.: PA5325, Abmart, People's Republic of China) overnight at 4°C. Then the sections were incubated for 50 minutes with anti-rabbit secondary antibody (1:200 dilution, Catlog No.: GB23303, Servicebio, People's Republic of China) followed with DAB solution incubation and hematoxylin staining. After dehydration, through degraded alcohol solutions, the sections were quickly sealed with neutral gum and covered with glass before the sections were observed under optical microscope. Immunoreactivity of candidate proteins was scored independently by two senior pathologists with blinding evaluation and protein expression was estimated by double score semi quantitative analysis. The grade of the score was determined by multiplying the score for staining intensity with the score for proportion of positive cells in tissue sections. Staining intensity was recorded as intensity score (0 = negative, 1 = weak, 2 = moderate and 3 = strong). As for the percentage scores, they were marked by positive cells (0 = 0%, 1 = 1%-25%, 2 = 26%-50%, 3 = 51%-75%, 4 = >75%). The staining score values are between 0 and 12 and scores of 0 to 7 were considered low expression for statistical analysis. The relationship between overall survival and IHC results of each patient was analyzed using the Kaplan-Meier survival plot and compared by Log rank tests.

Association Between STEAP1 and STEAP2 mRNA Levels and Immune Features of LUAD Patients

Tracking Tumor Immunophenotype (http://biocc.hrbmu.edu.cn/TIP/) was a web server for resolving tumor immunophenotype, which evaluated the status score of tumor-infiltrating immune cells based on TCGA-LUAD dataset. Using the sample ID, we obtained the normalized STEAP1 and STEAP2 expression data using R package TCGAbiolinks (v2.20.1). Correlation analysis was performed to show the correlation between immune infiltration levels and STEAP1 and STEAP2 mRNA levels using R package corrplot (v0.92). Meanwhile, TIMER2 (http://timer.cistrome.org/) was used to further explore the relationship between STEAP1 and STEAP2 expression and specific immune cells.

Mutation Feature Analysis

To observe the relation between the gene mutation status and the expression of STEAP1 and STEAP2 in LUAD, gene expression data from 508 LUAD patients and common gene mutation data were obtained from muTarget (https://www.mutarget.com/), a cancer biomarker/target discovery tool. Mann–Whitney analysis was used to test all the results and P-values < 0.001 were used as the cutoff value to screen out the most significant genes.

The Relationship Between STEAP1/2 Expression and Drug Resistance

The gene expression data and molecule/drug sensitivity data of 54 kinds of LUAD cell lines were obtained from Genomics of Drug Sensitivity in Cancer (GDSC, https://www.cancerrxgene.org/), a resource for therapeutic biomarker discovery in cancer cells. The expression of STEAP1 and STEAP2 was performed by Pearson correlation analysis with the small molecule/drug sensitivity (IC50). P value < 0.05 was used as the cut off value.

Statistical Analysis

All statistical analyses were performed using R software (v4.1.1). A two-sided $P < 0.05$ was recognized as statistical significance.

Results

Iron Metabolism Related Genes Showed Great Significance in LUAD Prognosis

One hundred and nineteen iron metabolism related genes were derived from MSigDB database. The univariate Cox regression analysis identified 20 iron metabolism related genes were significantly associated with overall survival of TCGA-LUAD patients ($P < 0.05$; Figure 1 and Supplementary Table 1). We analyzed the differences of the 20 genes' expression levels between LUAD tissues and adjacent normal tissues (Figure 2A), and the relationship between the 20 genes' expression level and the clinical features were presented as well as the PDCD1 and CD274 expression level in TCGA-LUAD patients (Figure 2B). Expression of 16 genes was significantly different between LUAD tissues and adjacent normal tissues, of which 7 genes, including STEAP1, SFXN1, STEAP2, GLRX3, CAND1, TTYH1 and ATP6V0E2, showed higher expression level in LUAD tumor compared to normal tissues.

GEPIA 2, an online survival analysis tool, was used to verify the result from TCGA-LUAD database. Ten genes including STEAP1, STEAP2, EGLN1, GLRX3, BTBD9, REP15, HRG, ATP6V0D2, SFXN1 and SLC46A1 also showed significant association with the prognosis of LUAD patients. Of note, STEAP1 showed the highest significance, followed by STEAP2 (Figure 3).

Higher Expression of STEAP1 and STEAP2 is Related to the Poorer Prognosis of LUAD

In order to explore the molecular mechanisms of tumor, it is essential to understand the interactions between these 20 genes in LUAD. The STRING database was used to draw the Protein-Protein Interaction Network, which showed that STEAP1 and STEAP2 are the hub genes among them (Figure 4A). Furthermore, the Pearson correlation coefficient

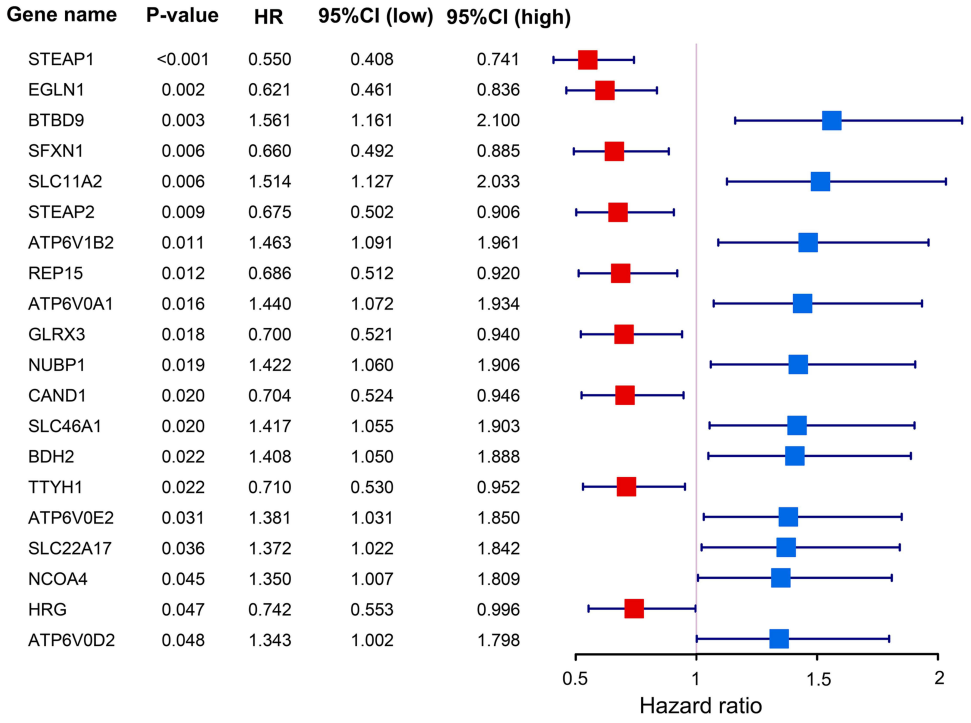

Figure 1 The forest plot exhibits the associations between iron metabolism related genes and prognosis in TCGA-LUAD cohort patients.
Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.

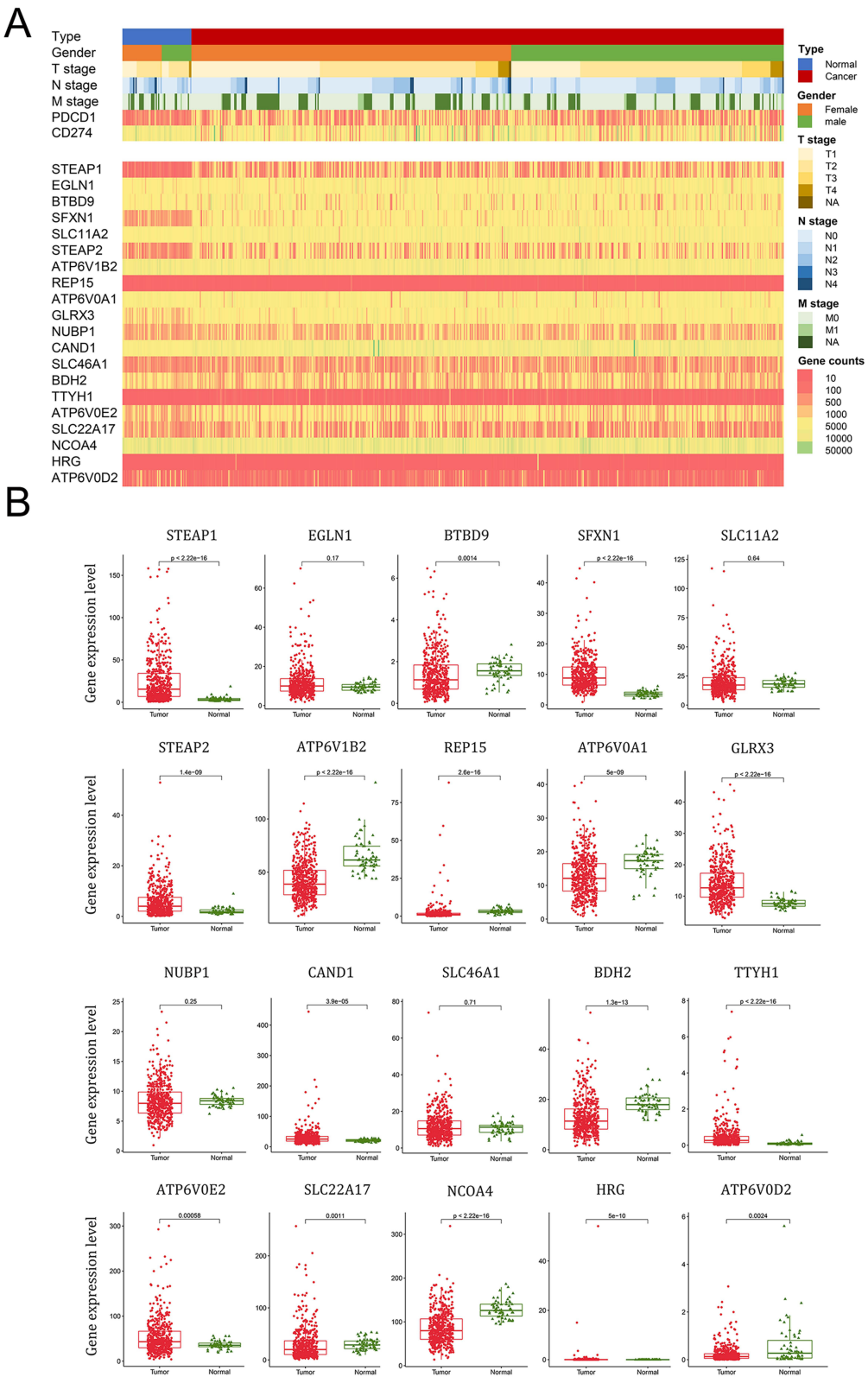

Figure 2 The expression levels of 20 iron metabolism related genes between LUAD and adjacent normal tissues in TCGA-LUAD dataset. (**A**) Heatmaps show the gene expression levels of 20 iron metabolism related genes and PDCD1 and CD274 in the TCGA-LUAD dataset. (**B**) The red points represent the normalized mRNA expression levels of iron metabolism related genes in 498 TCGA-LUAD tumor tissues while the green points represent the normalized mRNA expression level in 58 adjacent normal tissues. The P-values derived from the Student's *t*-tests are shown.

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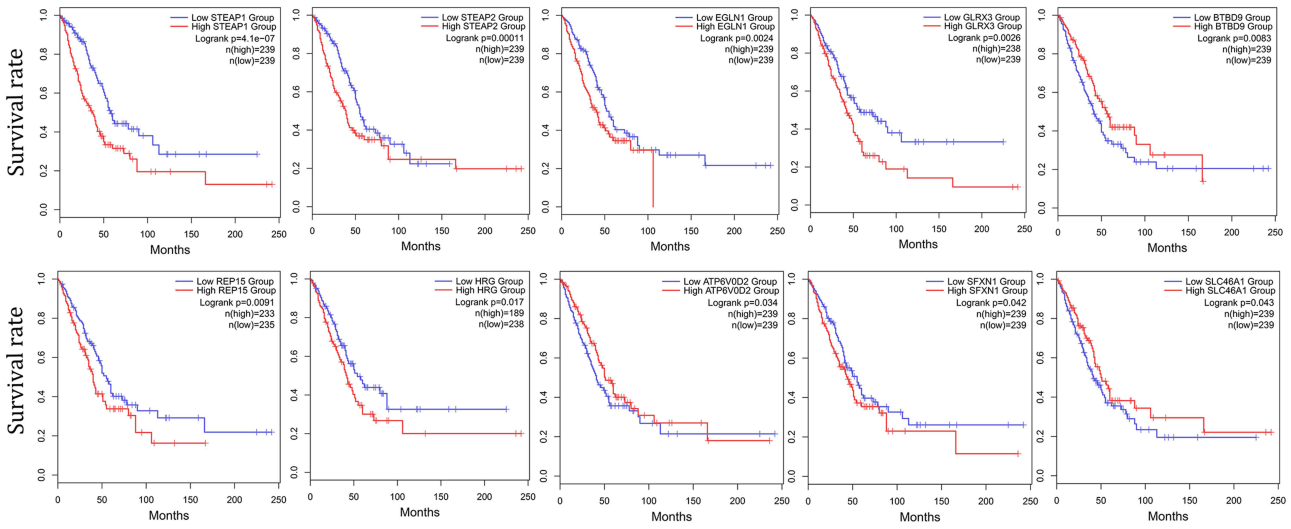

Figure 3 The Kaplan-Meier survival plots of the LUAD patients in GEPIA 2 database. The Kaplan–Meier plots of ten genes that are significantly associated with the prognosis of LUAD patients in GEPIA 2 database (N = 478). Patients were stratified by the median expression level of genes and compared using the Log rank tests.

between these 20 genes in LUAD showed that STEAP1 and STEAP2 hold the strongest correlation (Figure 4B) and their possible functions were further explored through the protein–protein interaction (PPI) network (Figure 4C). These results suggested that STEAP1 and STEAP2 may play central roles in the regulating of LUAD progression by the iron-metabolism related genes.

From April 2013 to August 2021, 30 newly diagnosed LUAD patients who had never received any treatment before, including 17 male subjects and 13 female subjects, aged from 45 years old to 78 years old, were recruited to the current study to evaluate the prognostic values of the STEAP1 and STEAP2 at the protein level. Of them, 13 subjects had formed the habit of smoking and 19 subjects were diagnosed with comorbidities including hypertension, diabetes and other lung disease. As for the TNM clinical stages, 16 subjects were categorized as I and II stages while 14 subjects were categorized as III and IV stages by pathologists. The follow-up period of these 30 patients ranged from 2 months to 101 months, with a median of 57 months. 12 patients succumbed to the disease, and 18 patients were alive in August 2021 (Supplementary Table 2). IHC staining results of 30 LUAD patients (Figure 5A) indicated that STEAP1 and STEAP2 proteins were detected in both cytoplasm and cytomembrane of the LUAD tissues (Figure 5B). 11 of 30 samples were diagnosed as STEAP1 high expression (staining score > 7; Supplementary Table 2) and 16 of 30 samples were diagnosed as STEAP2 high expression (staining score > 7; Supplementary Table 2). We found that the patients with higher STEAP1 protein expression showed shorter overall survival time compared to subjects with low expression of STEAP1 (P = 0.0019) and patients with higher STEAP2 protein expression were also associated with poorer overall survival (P = 0.049) (Figure 5C). Meanwhile, no significant difference in the patients' characteristics between STEAP1/2-high and STEAP1/2-low groups was noticed (Supplementary Tables 3 and 4), which further demonstrated that STEAP1 and STEAP2 expression level could be the independent risk factors for LUAD patients.

Correlation Between Immune Cell Infiltration Levels and STEAP1 and STEAP2 mRNA Levels

Tumor immune microenvironment is composed of a variety of cellular subtypes, extracellular matrix components, blood system and oxidative free radicals, which is closely associated with the tumor initiation and progression. To investigate the effects of iron metabolism related genes on tumor microenvironment we analyzed the correlations between immune activity scores and immune cell infiltration levels with the expression levels of these 20 genes in 594 TCGA-LUAD tumor tissues based on the TIP database. We found that the mRNA levels of STEAP1 and STEAP2 are positively correlated with most recruited immune cell levels, including CD8+ T cells, Th1 cells, dendritic cells, macrophage, monocyte, neutrophil, NK cells, eosinophil, and basophil cells but negatively correlated with CD4+ T cells, which play important roles in tumor immunity through modulating dendritic cells or

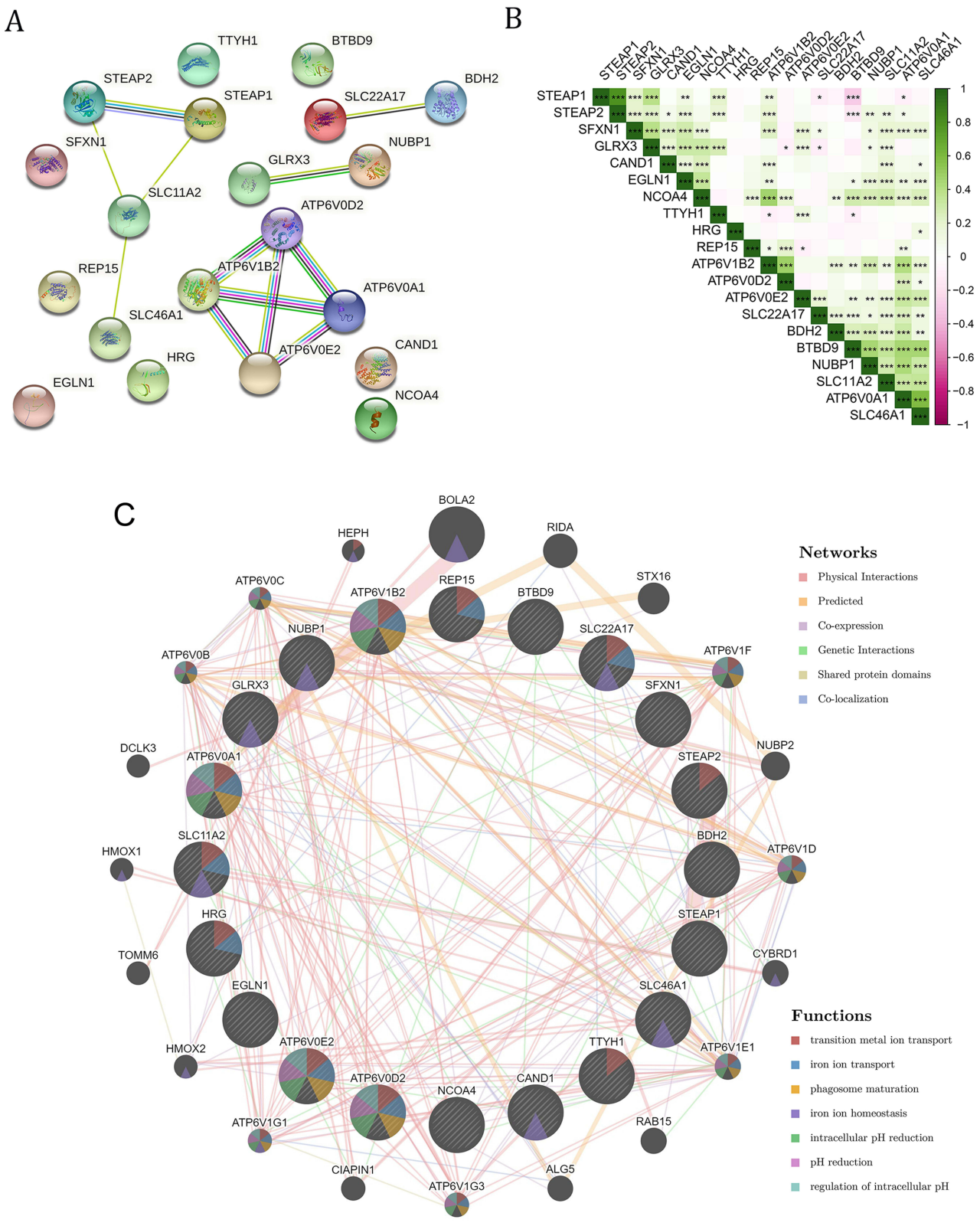

Figure 4 The protein-protein interaction (PPI) network and gene expression correlations between 20 genes. (**A**) The protein–protein interaction (PPI) network inferred by the STRING database The nodes represent proteins and the edges represent protein-protein interactions. (**B**) The heatmap shows the correlation coefficient between genes in 498 TCGA-LUAD tumor tissues. ***P < 0.001, **P < 0.01, *P < 0.05. (**C**) The PPI network of 20 iron metabolism related genes visualized by the GeneMANIA database.

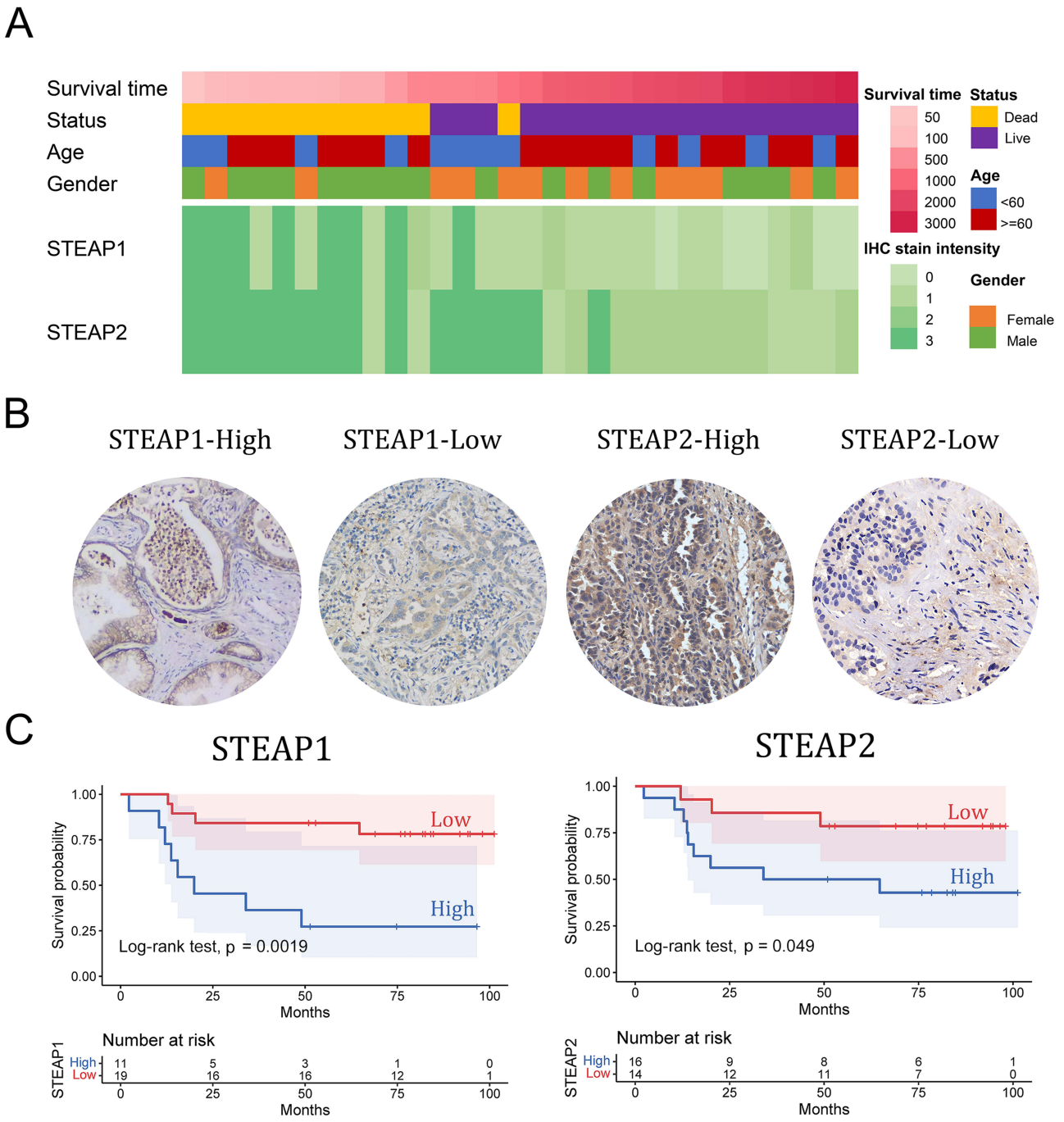

Figure 5 Associations between STEAP1 and STEAP2 protein levels and the prognosis in LUAD patients. (**A**) Heatmaps show the IHC staining results of STEAP1 and STEAP2 of 30 LUAD patients as well as corresponding clinical features. (**B**) Representative immunohistochemistry images of LUAD tissue with higher or lower STEAP1/2 expression level (×200). (**C**) The Kaplan–Meier plots of the overall survival in LUAD patients with higher or lower STEAP1 and STEAP2 expression levels according to IHC staining scores. P-values derived from the Log rank tests were labeled.

stimulating other pro-inflammatory myeloid cells to enhance immune activities (Figure 6A). CD4 and CD8 T cells can be subdivided into different populations with distinct functions. STEAP1 is negatively correlated with CD4 naïve T cells and CD8 memory T cells but positively with CD4 memory T cells and CD8 effector T cells. For STEAP2, the expression level is negatively correlated with CD8 memory T cells but positively with CD4 memory T cells and CD8 effector T cells (Figure 6B). Also, to explore the association between the expression of STEAP1, STEAP2 and PD-1, PD-L1 molecules, correlation analysis between the expression level of STEAP1 and STEAP2 and the expression level of PDCD1 and CD274 was performed using the data from

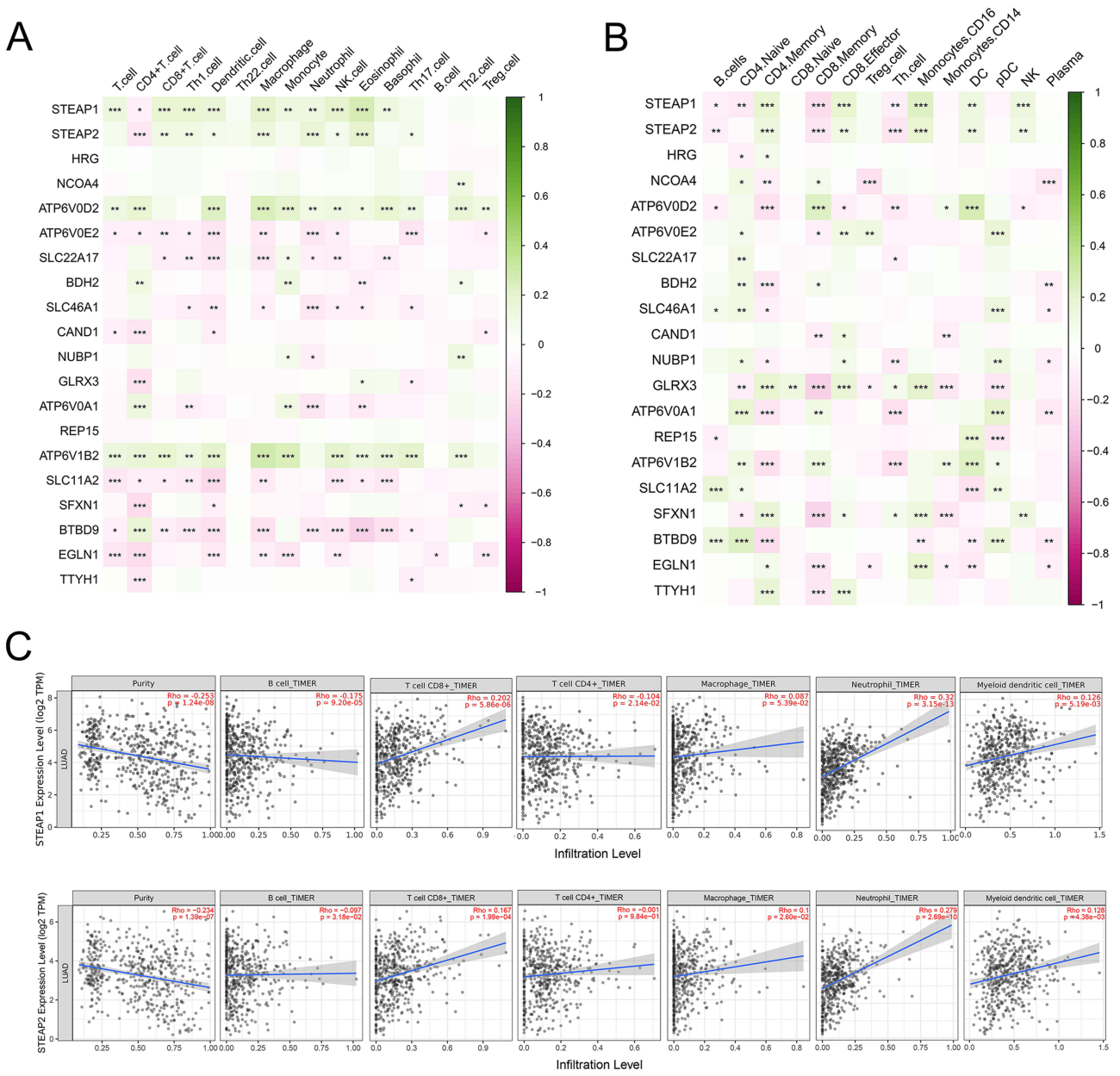

Figure 6 Correlations between 20 iron metabolism related genes and immune activities in TCGA-LUAD tissues. (**A**) Correlations between 20 iron metabolism related genes and immune activity scores (cell recruiting) in TCGA-LUAD tumor tissues. (**B**) Correlations between 20 iron metabolism related genes and immune cell infiltration (relative proportion of tumor-infiltration immune cells) in TCGA-LUAD tumor tissues. ***P < 0.001, **P < 0.01, *P < 0.05. (**C**) The correlation of STEAP1 and STEAP2 expression level with immune cells validated by the TIMER database.

TCGA-LUAD dataset. Statistical analysis results showed significant association between STEAP1 and PDCD1 (r = 0.22, P = 7.67e-7), STEAP1 and CD274 (r = 0.39, P = 4.54e-19), STEAP2 and PDCD1 (r = 0.21, P = 3.83e-6) and STEAP2 and CD274 (r = 0.34, P = 2.47e-15).

Relationship Between Gene Mutation and STEAP1 and STEAP2 Expression Level

To clarify whether the gene mutation status may influence the expression of STEAP1/2 in LUAD, the gene mutation status of 508 TCGA-LUAD samples were obtained from the muTarget database. At the gene mutation frequency threshold > 2% and the significance level of P < 0.001, we noticed 10 types of gene mutations showed significant association with STEAP1 expression level (Figure 7A), while 7 others were significantly associated with the expression

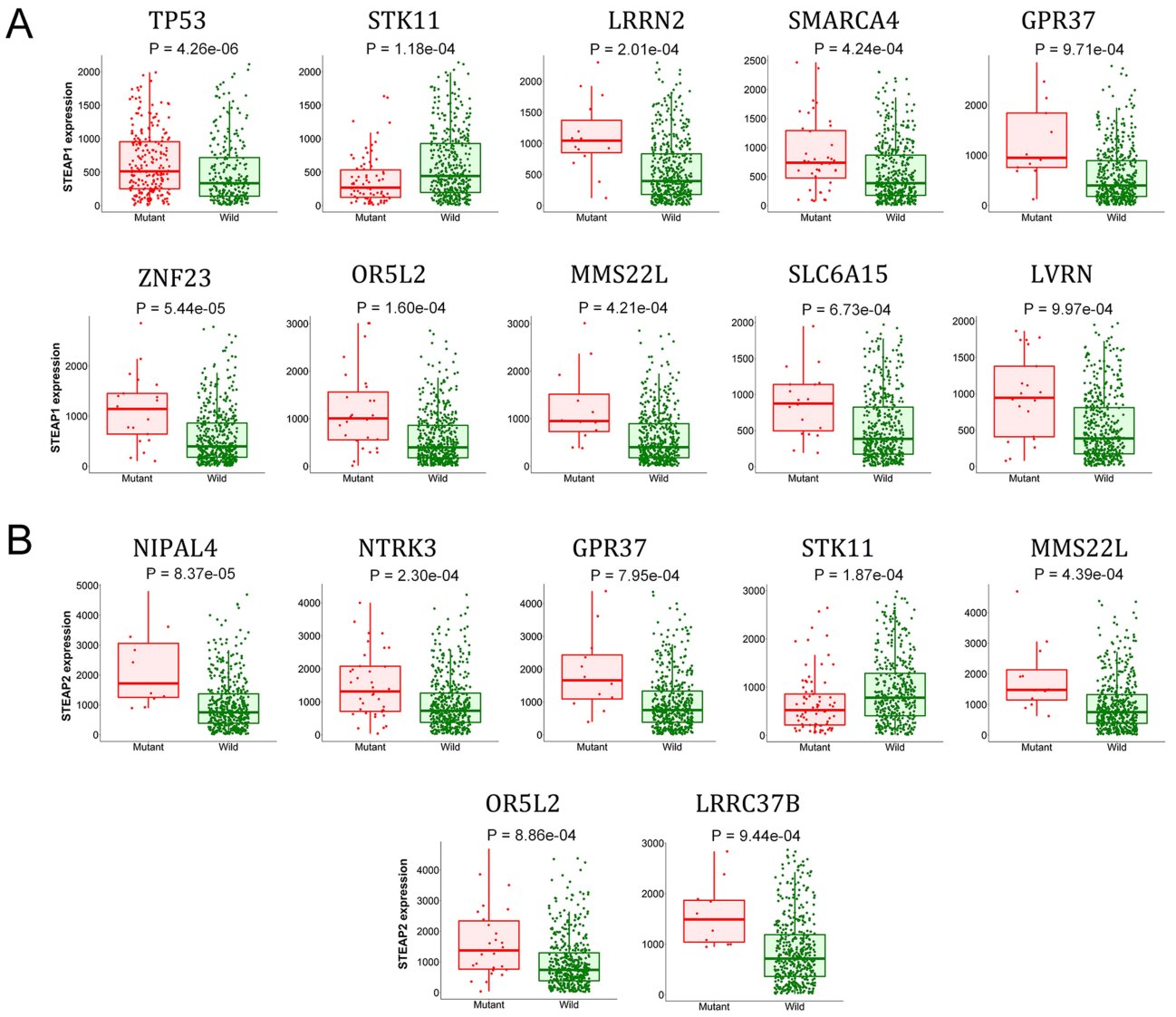

Figure 7 Differential STEAP1 and STEAP2 gene expression levels in TCGA-LUAD tissues with or without distinct gene mutations. The red point shows the mRNA levels in samples with indicated gene mutation and the green point represents tissues without gene mutation.

of STEAP2 (Figure 7B). Among them, TP53 mutation status showed the most significant association with expression of STEAP1 and patients with TP53 mutation were associated with higher expression of STEAP1 (Figure 7A), indicating that TP53 mutation may influence the prognosis of LUAD patients through regulating the iron metabolism.

Associations Between mRNA Level of STEAP1/2 and Drug Resistance in LUAD Cell Lines

To explore whether the expression level of STEAP1 and STEAP2 will affect the drug sensitivity of LUAD cells we evaluated the correlation between STEAP1 and STEAP2 mRNA levels in 54 LUAD cell lines with the IC50 value of 181 small molecules/drugs. Among them, Navitoclax, Lapatinib, JAK and AZD showed significant correlation with the mRNA level of STEAP1. Furthermore, IC50 value of 13 drugs including Lapatinib_1558 and JAK were positively correlated with the mRNA level of STEAP2 (Figure 8). These results suggested that STEAP1 and STEAP2 may influence prognosis through regulating the drug sensitivity of LUAD cells.

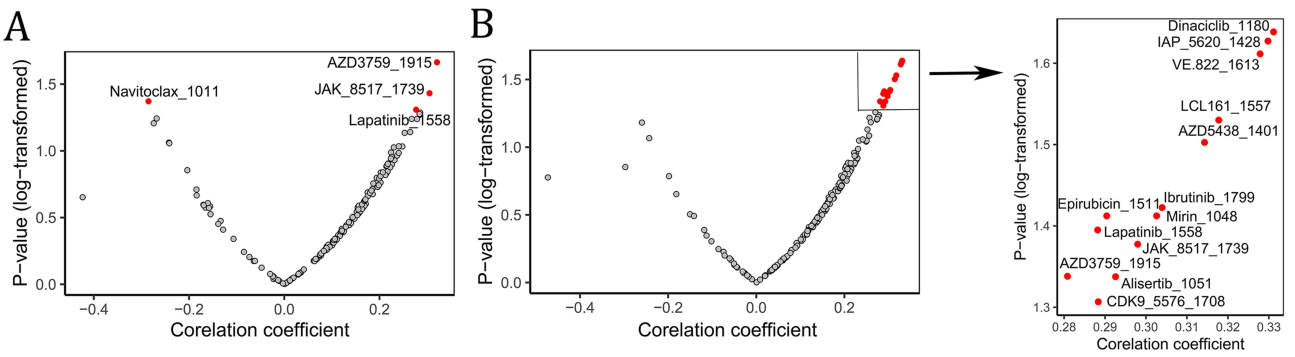

Figure 8 Correlations between STEAP1 and STEAP2 gene expression levels and molecule/drug sensitivity in LUAD cell lines. The X-axis represents the Pearson correlation coefficient between genes STEAP1 (**A**) and STEAP2 (**B**) and IC50 and 181 compounds in 54 kinds of LUAD cell lines derived from GDSC database. The corresponding P-values are shown on the Y-axis. Each dot shows individual compound and the significant dots ($P < 0.05$) are colored red and labeled.

Discussion

Lung cancer is usually influenced synthetically by many environmental, genetic and metabolic factors.[22] Enhanced energy metabolism rate is a characteristic of tumor cells, which is closely associated with the corresponding protein expression levels. Recent studies further facilitate our understanding of the mechanisms involved in development and progression of lung cancer, which may uncover novel prognostic biomarkers or therapeutic targets. For example, GLUT-1 is strongly involved in tumor metabolism and p16 could lead to G0 phase cell cycle arrest and induce tumor cell apoptosis and they were verified to be abnormally expressed in malignant lung tissues and could serve as potential biomarkers of lung cancer evolution and aggressiveness.[23] HIF-1a, influenced by exogenous factors such as Nicotine, are able to activate angiogenesis signaling pathways.[22] Protein expression related techniques are closely related to the metabolic of lung tumors and have become new tools for diagnosis, prognosis and treatment of it. In this study, we analyzed the data obtained from the TCGA and GEPIA 2 databases, and investigated the prognostic values of iron metabolism related genes in LUAD patients. Furthermore, we found the expression of STEAP1 and STEAP2 are associated with the level of immune cell infiltration, gene mutation and drug resistance, uncovering the mechanisms for the associations between STEAP1 and STEAP2 expression levels and the prognosis of LUAD patients.

Multiple iron metabolism related genes were differentially expressed between LUAD tissues and normal tissues, which suggested that some iron metabolism related proteins may be involved in the progression of LUAD. The iron metabolism genes STEAP1 and STEAP2 act as the hub genes according to the PPI network analysis. STEAP proteins are cell surface antigens and are mainly expressed in bladder cancer, colon cancer and lung cancer.[24–27] As the ion channel, receptor, or transporter,[26,28] they promote tumor cell proliferation and inhibit apoptosis.[29–31] In a current study, Liu et al explored the prognostic value of STEAP1 and STEAP2 using the data of Kaplan-Meier plotter database, and for patients with NSCLC, results showed STEAP1 protein overexpression was significantly correlated with poor prognosis of lung cancer, and STEAP2 protein downregulation was significantly correlated with poor prognosis of lung cancer. However, after dividing lung cancer patients into different stages, consistent significant results were found in stage I lung cancer, and for patients with stage II and III lung cancer, the expression and prognosis of STEAP1 and STEAP2 were not statistically significant.[19] Thus, further exploration was needed for the prognostic value of STEAP1 and STEAP2 in different subtypes of lung cancer. We validated the prognostic value of STEAP1 and STEAP2 in LUAD tissues using IHC. As predicted by the public TCGA-LUAD data analysis result, the IHC results suggested that STEAP1 and STEAP2 protein overexpression were significantly correlated with poor prognosis of LUAD patients.

There may be several underlying mechanisms of the prognostic effects of STEAP1 and STEAP2 in LUAD patients. Expression of STEAP1 and STEAP2 were correlated with multiple aspects of immune cell infiltration. Tumor infiltrating lymphocytes have been demonstrated to be an independent predictor of cancer prognosis.[32] It can affect downstream innate and adaptive immune responses by influencing the macrophage effector functions.[33] For example, the activity of the cancer-immunity cycle is a direct manifestation of immune regulatory system, and STEAP1 and STEAP2 were correlated with some steps in cancer–immunity cycle including releasing of the cancer cell antigens,

trafficking of immune cells to tumors, infiltration of immune cells into tumors and killing of cancer cells.[33,34] Previous studies have shown that the imbalance of STEAP1 and STEAP2 might lead to the immunosuppressive microenvironment of glioma by affecting the cancer immune cycle, immune infiltration and phenotype.[35] A growing body of evidence has shown that STEAP1 and STEAP2 influence the invasive behavior and oxidative stress phenotype, and STEAP1- derived peptides are immunogenic.[36,37] In our study, higher STEAP1 and STEAP2 expression levels were positively correlated with multiple aspects of immune cell infiltration, especially the innate immune cells such as eosinophils, macrophages and NK cells, indicating that the poorer prognosis of LUAD patients with high iron metabolism may be due to the stronger immunosuppression. Encoded by PDCD1 gene and CD274 gene respectively, the PD-L1/PD1 and PDL1 signaling pathway could inhibit the immune cytotoxicity effects of T lymphocytes thus facilitating the immune escape of tumor cells and promoting the occurrence and development of tumors.[38] Inhibitors of the PD-1 axis have altered the treatments of non-small-cell lung cancer (NSCLC) over the last decade.[39] In the current study, we found that the expression level of STEAP1 and STEAP2 were positively associated with the expression of PD-1 and PD-L1 levels in TCGA-LUAD dataset, indicating the potential mechanisms of the negative prognostic values of STEAP1 and STEAP2.

Tumors often occur with the accumulation of genetic mutations, which help them to escape from the immune system, leading to uncontrolled growth and metastasis. Our study demonstrated that TCGA-LUAD patients with higher expression of STEAP1 and STEAP2 are significantly associated with the mutation status of multiple genes, especially TP53 and STK11. TP53, the most commonly mutated gene in TCGA-LUAD, is a tumor suppressor gene which triggers a series of alterations in the progression and clinical outcome of TCGA-LUAD.[40] As a transcription factor, tumor suppressor p53 exerts its tumor function primarily via its transcriptional modulation of its downstream target genes.[41] p53 is involved in regulation of a wide range of cancer-associated pathways, and mutant p53 potentially triggers chromosomal/genomic instability which is frequently related to poor prognosis in LUAD.[42] After TP53 and KRAS, STK11 is the third most commonly mutated gene in LUAD, which encodes the protein LKB1.[43] In addition, the canonical tumor-suppressive role of STK11 involves the activation of AMPK-related kinases, inactivation of LKB1 is associated with an inert tumor immune microenvironment and the progression of lung cancer.[44] These data suggested that mutations in these genes may regulate the tumor development and progression through regulating the gene expression levels of STEAP1 and/or STEAP2.

Drug resistance often leads to poorer prognosis in LUAD patients. Many anticancer drugs were found to play roles by inducing iron death, such as metformin, artemisinin derivatives, Sorafenib, and Triapine.[45,46] In this study, we noticed that the LUAD cell lines with higher STEAP1 and STEAP2 expression levels showed significant association with the inhibitory activities of anticancer compounds. Overexpression of STEAP1 in LUAD cell lines resulted in the reduction of therapy effects of Lapatinib (targeting EGFR signaling pathway), JAK (targeting JAK1, JAK2 pathway) and AZD (targeting EGFR signaling pathway). Similarly, the IC50 of 13 molecules/drugs including JAK and AZD were positively correlated with the expression level of STEAP2. We speculated that the expression of STEAP1 and STEAP2 in LUAD may lead to adverse prognosis of LUAD through mediating resistance to multiple anti-cancer compounds.

There are several limitations that should be acknowledged. First, we explored the prognostic values of iron metabolism related genes in LUAD and validated the prognostic effects of STEAP1 and STEAP2 through IHC staining. Whether other genes were significantly associated with LUAD patients' prognosis at the protein level is unknown. Second, we identified the correlations between STEAP1 or STEAP2 expression levels and the resistant activities to anti-cancer compounds, which need to be validated with biological experiments. Finally, whether STEAP1 and STEAP2 could serve as promising targets in LUAD patients need to be further addressed.

Overall, we identified the association between 119 iron metabolism related genes and the prognosis of LUAD patients. Data from multiple databases indicated that STEAP1 and STEAP2 protein expression levels were associated with the prognosis of LUAD patients. Furthermore, the analysis results of immune cell infiltration level, gene mutation status, and drug resistance showed that iron metabolism related genes may influence the prognosis of LUAD through multiple aspects; however, detailed mechanisms need further exploration and validation.

Abbreviations

LUAD, lung adenocarcinoma; PPI, protein-protein interaction; IHC, immunohistochemistry; STEAP1, six-transmembrane epithelial antigen of the prostate-1; STEAP2, six-transmembrane epithelial antigen of the prostate-2; TME, tumor microenvironment.

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Disclosure

All authors declared that there are no conflicts of interest in this study.

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