Original Article

Bioinformatics analysis reveals *SOD1* is a prognostic factor in lung adenocarcinoma

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Background: Lung cancer is a major cause of cancer-related deaths worldwide. Unfortunately, non-small cell lung cancer (NSCLC) often lacks clear clinical symptoms and molecular markers for early diagnosis, which can hinder the initiation of timely treatments. In this study, we conducted an extensive bioinformatics analysis of copper-zinc superoxide dismutase (SOD1), a molecule linked to lung adenocarcinoma (LUAD) to enhance early detection and treatment approaches for this condition.

Methods: A bioinformatics analysis was conducted using a dataset from The Cancer Genome Atlas (TCGA) database. Several analytical methods, such as a differential expression analysis, a Kaplan-Meier survival analysis, a clinicopathological analysis, an enrichment analysis, protein-protein interaction (PPI) network construction using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database, and an immunoreactivity analysis of *SOD1* expression in LUAD using TIMER were employed. We further validated the expression of *SOD1* in LUAD through *in vitro* experiments using quantitative polymerase chain reaction (qPCR) and Western blot.

Results: Our findings indicate that LUAD tissues exhibited significantly higher expression levels of *SOD1* than healthy tissues. The univariate Cox analysis showed that the elevated level was linked to unfavorable overall survival (OS) rates. Further, the Cox regression analysis of multiple variables suggested that elevated *SOD1* expression levels acted as an autonomous prognosticator for unfavorable OS. We also conducted Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses, and a gene set enrichment analysis (GSEA) and observed differential pathway enrichment among patients with high *SOD1* expression. In addition, a correlation between *SOD1* and immune cell infiltration was found. The *in vitro* experiments confirmed that *SOD1* expression was upregulated in LUAD.

Conclusions: *SOD1* could serve as a reliable prognostic indicator in individuals diagnosed with LUAD. Our findings may prove valuable in the development of therapeutic and prognostic markers for LUAD. The potential clinical utility of *SOD1* in LUAD requires further investigation.

Keywords: Copper-zinc superoxide dismutase (SOD1); lung adenocarcinoma (LUAD); bioinformatics

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Submitted Aug 10, 2024. Accepted for publication Sep 13, 2024. Published online Oct 14, 2024.
doi: 10.21037/tcr-24-1400
View this article at: https://dx.doi.org/10.21037/tcr-24-1400

Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide. Of the 1.8 million individuals diagnosed with lung cancer each year, 1.6 million die from the disease (1). With only 10–20% of patients surviving for 5 years or more, lung cancer has a low survival rate (2). The risk factors associated with lung cancer include smoking, exposure to smoke or toxic occupational environments, chronic lung disease, and a family history of the disease (3). Despite being highly prevalent in China, non-small cell lung cancer (NSCLC) often goes untreated in the early stages due to a lack of noticeable clinical symptoms (3-5). Significant progress has been made in treating various forms of cancer, but lung cancer remains difficult to treat due to an absence of effective therapies (6). Consequently, new biomarkers need to be identified to facilitate early diagnosis, assist in prognosis evaluations, and guide treatment development.

Copper-zinc superoxide dismutase (SOD1) is an antioxidant enzyme discovered in 1969 that resides in the cytosol and catalyzes the conversion of superoxide to oxygen and hydrogen peroxide. It has also been shown to activate nuclear gene transcription or act as a RNA-binding protein (7). *SOD1* has been implicated in various diseases, including amyotrophic lateral sclerosis (ALS) and cancer. *SOD1* plays a crucial role in familial ALS, with genetic mutations and dysfunction contributing to its pathogenesis (8). There is growing evidence that *SOD1* also plays a significant role in cancer. Notably, *SOD1* has been shown to be overexpressed in many types of cancer, including lung (9) and primary breast cancer (10). However, the role of *SOD1* in lung adenocarcinoma (LUAD) remains unclear.

The main objective of this study was to examine *SOD1* expression in LUAD. Initially, we analyzed the differences in the expression of *SOD1* in tumor and normal tissues in LUAD patients and assessed the correlation between *SOD1* expression and overall survival (OS). Additionally, using univariate and multivariate Cox regression models, we investigated whether the expression levels of *SOD1* were correlated with any clinicopathological parameters. To uncover the biological mechanisms of *SOD1*, we conducted a Gene Ontology (GO) analysis, a Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, and a gene set enrichment analysis (GSEA). We also examined the link between *SOD1*, immune infiltration, and their effect on the development of LUAD. We present this article in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1400/rc).

Highlight box

Key findings

- Our findings indicate that lung adenocarcinoma (LUAD) tissues exhibit significantly higher levels of copper-zinc disulfide sulfurase (*SOD1*) expression than healthy tissues.

What is known, and what is new?

- *SOD1* is known to be related to amyotrophic lateral sclerosis.
- This study showed that *SOD1* was also associated with LUAD.

What is the implication, and what should change now?

- *SOD1* could serve as a reliable prognostic indicator in individuals diagnosed with LUAD. However, the potential clinical utility of *SOD1* in LUAD requires further investigation.

Methods

Clinical data

Messenger RNA (mRNA) expression data from 598 samples were acquired from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/) using the HTSeq-FPKM workflow type. The dataset also included clinical the information of patients diagnosed with LUAD. In total, 598 patients with LUAD and appropriate clinical features were included in this research. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

SOD1 expression in individuals diagnosed with LUAD

The ggplot2 R package (version 3.3.3; https://cran.r-project.org/web/packages/ggplot2/index.html) was used to explore the level of *SOD1* expression in patients with LUAD and compare it in both tumor and normal tissues, and tumor and paracancerous tissues.

Association between SOD1 and survival

To analyze the association between OS and various clinicopathological parameters, we used the survminer R package (version 0.4.9) in combination with the survival R package (version 3.2-10) to conduct the Cox regression analyses. The parameters included age (>65 *vs.* ≤65 years), gender (male *vs.* female), smoking status (yes *vs.* no), smoking age (≥40 *vs.* <40 years), race (White *vs.* Asian/Black or African American), pathological stage (stage I *vs.* II *vs.* III *vs.* IV), primary treatment outcome [partial response (PR) *vs.* complete response (CR) *vs.* progressive disease (11) *vs.* stable disease (SD)], residual tumor (R0 *vs.* R1 *vs.* R2), T stage (T1 *vs.* T2 *vs.* T3 *vs.* T4), N stage (N0 *vs.* N1 *vs.* N2 *vs.* N3), and M stage (M1 *vs.* M0).

Functional enrichment analysis

In our study, we used the ggplot2 R package (version 3.3.3) and clusterProfiler R package (version 3.14.3) to identify genes that were strongly correlated with *SOD1* expression levels in LUAD. KEGG and GO enrichment analyses were then performed to predict the functions of *SOD1* and its co-expressed genes in the TCGA-LUAD dataset. The identification of shared protein functions provides valuable insights into molecular mechanisms underlying cellular processes. To this end, we used a search tool to effectively investigate the protein-protein interaction (PPI) network, which was built using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://string-db.org). By examining the central nodes in the PPI network, we identified potential genes that might exert crucial functions in physiological regulation or act as central proteins.

GSEA

Expression patterns were compared among groups with high and low levels of *SOD1* expression to identify potential genes that showed differential expression using the DESeq2 R package (version 1.26.0). A GSEA (http://www.gsea-msigdb.org/gsea/index.jsp) was conducted on a set of approximately 56,493 differentially expressed genes (DEGs). The present investigation used the Molecular Signatures Database Collection from the clusterProfiler R package (version 3.14.3) to perform the GSEA. The study sought to identify pathway differences in high *SOD1* expression groups in LUAD, with *SOD1* expression level serving as the phenotype label.

Analysis of immune cell infiltration

The study employed the TIMER (http://cistrome.dfci.harvard.edu/TIMER/) correlation module to evaluate possible associations between *SOD1* expression and the infiltration of immune cells in tumors. All clinical data and baseline information in the TIMER database are derived from the TCGA database (*Table 1*). The analysis included 28 types of immune cells, such as macrophages, neutrophils, T follicular helper (Tfh) cells, eosinophils, natural killer (NK) cells, NK cluster of differentiation (CD)56 bright cells, NK CD56 dim cells, T helper (Th)17 cells, gamma delta T (Tgd) cells, and Th2 cells. The research calculated Spearman's rank correlation coefficients to determine the magnitude of the relationship between the expression of *SOD1* and the level of infiltration by immune cells in the tumors. To gain a more comprehensive understanding of the influence of *SOD1* on the immune microenvironment, we assessed 539 tumor samples and divided the samples into two groups. Through the application of a significance level threshold of <0.05, we determined the particular categories of lymphocytes influenced by *SOD1* expression.

Real-time quantitative polymerase chain reaction (RT-qPCR) of cell lines

In this study, total RNA was extracted using TRIzol reagent, and mRNA was reverse transcribed with Oligo-dT primers. RT-qPCR was performed using the Step One Plus Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA), with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal reference. The following RT-qPCR primers were used in this study: *SOD1* forward primer, AAAGATGGTGTGGCCGATGT; *SOD1* reverse primer, CAAGCCAAACGACTTCCAGC; GAPDH forward primer, CTGGGCTACACTGAGCACC; and GAPDH reverse primer, AAGTGGTCGTTGAGGGCAATG.

Western blot assay

We used BEAS-2B human bronchial epithelial cells, NCI-H1395 human LUAD cells, NCI-H157 human NSCLC carcinoma cells, and Calu-3 human LUAD cells, all sourced from iCell (Shanghai, China). Anti-*SOD1* was

Table 1 Clinical characteristics of the LUAD patients

Characteristics	Low expression of *SOD1* (n=269)	High expression of *SOD1* (n=270)	P value
Gender (n=539), n (%)			0.63
Female	147 (27.3)	142 (26.3)	
Male	122 (22.6)	128 (23.7)	
Age (n=520), n (%)			0.66
≤65 years	127 (24.4)	130 (25.0)	
>65 years	135 (26.0)	128 (24.6)	
Race (n=472), n (%)			0.10
Asian	1 (0.2)	7 (1.5)	
Black or African American	29 (6.1)	26 (5.5)	
White	205 (43.4)	204 (43.2)	
Number of packs smoked per year (n=369), n (%)			0.57
<40	98 (26.6)	90 (24.4)	
≥40	89 (24.1)	92 (24.9)	
Residual tumor (n=374), n (%)			0.02
R0	177 (47.3)	180 (48.1)	
R1	3 (0.8)	10 (2.7)	
R2	0 (0.0)	4 (1.1)	
Pathologic M stage (n=390), n (%)			<0.001
M0	184 (47.2)	181 (46.4)	
M1	4 (1.0)	21 (5.4)	
Pathologic N stage (n=523), n (%)			0.13
N0	186 (35.6)	164 (31.4)	
N1	41 (7.8)	56 (10.7)	
N2	31 (5.9)	43 (8.2)	
N3	1 (0.2)	1 (0.2)	
Pathologic T stage (n=536), n (%)			0.50
T1	93 (17.4)	83 (15.5)	
T2	146 (27.2)	146 (27.2)	
T3	22 (4.1)	27 (5.0)	
T4	7 (1.3)	12 (2.2)	

LUAD, lung adenocarcinoma; *SOD1*, copper-zinc superoxide dismutase.

purchased from AFFINITY (Nanjing, China; catalog No. AF5198, 1:1,500), and anti-ACTIN was purchased from Proteintech (Wuhan, China; catalog No. 20536-1-AP, 1:5,000). The cell or tissue samples were lysed using radioimmunoprecipitation assay buffer containing protease inhibitor, and the insoluble material was removed by centrifugation at 4 ℃ at 12,000 g for 30 minutes to obtain the supernatant as the total protein sample. The protein concentration in the lysate was determined using the bicinchoninic acid protein assay, and the protein samples

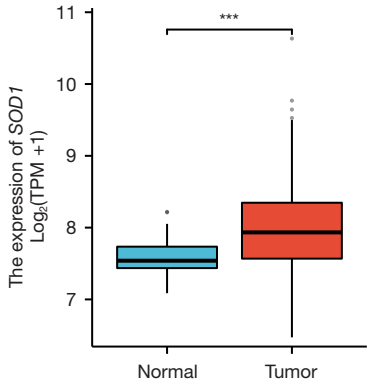

Figure 1 Levels of *SOD1* expression in normal and tumor tissues. ***, P<0.001. *SOD1*, copper-zinc superoxide dismutase; TPM, transcript per million.

were adjusted and stored at −80 ℃. Polyacrylamide gels for sodium dodecyl-sulfate polyacrylamide gel electrophoresis were prepared to separate the protein samples. Following electrophoresis, the proteins were transferred from the gel to polyvinylidene fluoride membranes using a wet transfer system. The membranes were then blocked with bovine serum albumin (BSA) solution to minimize nonspecific binding for 1.5 hours. Primary antibodies (*SOD1* and ACTIN) were diluted in BSA solution and added to the samples, and then incubated overnight at 4 ℃. After washing the membranes with Tris-buffered saline with Tween (TBST) to remove unbound antibodies, they were then incubated with a horseradish peroxidase- or alkaline phosphatase-conjugated secondary antibody matching the host species of the primary antibody (secondary antibody) for 2 hours. Following another round of washing with TBST, chemiluminescent imaging was performed using the AI600 chemiluminescent imaging system, and a band intensity analysis was conducted using ImageJ software.

Statistical analysis

The expression of SOD1 in LUAD was analyzed using the Wilcoxon rank-sum test. Survival analysis was performed using Kaplan-Meier survival curves. Functional enrichment analysis related to LUAD and SOD1 was conducted using multivariate Cox regression analysis, while differential analysis was carried out using analysis of variance (ANOVA). All statistical analyses were performed using R software (version 4.2.1) and SPSS software (version 26.0). P<0.05 was considered statistically significant.

Results

Baseline characteristics of patients

We obtained data from a cohort of 539 individuals with LUAD that were generated by TCGA research network. Patient characteristics, such as gender, age, race, smoking status (including the number of packs smoked per year), residual tumor, primary therapy outcome, and pathologic tumor-node-metastasis (TNM) stage were recorded (*Table 1*).

Expression status of SOD1 in LUAD

To assess the statistical significance of the two distributions, the Wilcoxon rank-sum test was used. The results showed that the *SOD1* expression levels were markedly more elevated in the tumor tissues than the normal tissues (P<0.001) (*Figure 1*).

Association analysis between SOD1 expression and clinicopathological characteristics

Subsequently, an exploration was conducted to examine the correlation between *SOD1* expression in tumors and clinicopathologic variables in LUAD cancer (*Table 2*).

Association between SOD1 and survival

Figure 2 illustrates the results of the Kaplan-Meier survival analysis, which revealed a notable relationship between elevated *SOD1* expression and an unfavorable prognosis (P<0.001). Univariate and multivariate Cox regression models were used to examine the prognostic factors of LUAD. The univariate Cox regression analysis indicated a statistically significant relationship between elevated levels of *SOD1* expression and unfavorable OS outcomes (P≤0.001). As *Table 3* and *Figure 3* show, the univariate Cox regression analysis revealed that the *SOD1* gene expression was an autonomous predictive factor for OS among LUAD patients (P<0.001).

SOD1-related functional enrichment analysis

GO term and KEGG pathway analyses were performed to investigate the possible biological functions of *SOD1*. The GO annotation analysis identified the following nine categories that exhibited a significant positive association with elevated levels of *SOD1* expression: the quinone metabolic process, the cellular ketone metabolic process,

Table 2 Logistic regression analysis of the relationship between *SOD1* expression and clinical characteristics

Characteristics	Total, n	OR (95% CI)	P value
Age (>65 *vs.* ≤65 years)	520	0.926 (0.657–1.306)	0.66
Gender (male *vs.* female)	539	1.086 (0.774–1.524)	0.63
Smoker (no *vs.* yes)	525	1.222 (0.752–1.985)	0.42
Number packs smoked per year (≥40 *vs.* <40)	369	1.126 (0.748–1.693)	0.57
Race (White & Black or African American *vs.* Asian)	472	0.140 (0.017–1.150)	0.07
Residual tumor (R1 & R2 *vs.* R0)	374	4.589 (1.297–16.241)	0.01
Pathologic T stage (T2 & T3 & T4 *vs.* T1)	536	1.185 (0.826–1.699)	0.36
Pathologic N stage (N1 & N2 & N3 *vs.* N0)	523	1.554 (1.076–2.244)	0.01
Pathologic M stage (M1 *vs.* M0)	390	5.337 (1.797–15.853)	0.003
Pathologic stage (stage II & stage III & stage IV *vs.* stage I)	531	1.502 (1.065–2.120)	0.02

SOD1, copper-zinc superoxide dismutase; OR, odds ratio; CI, confidence interval.

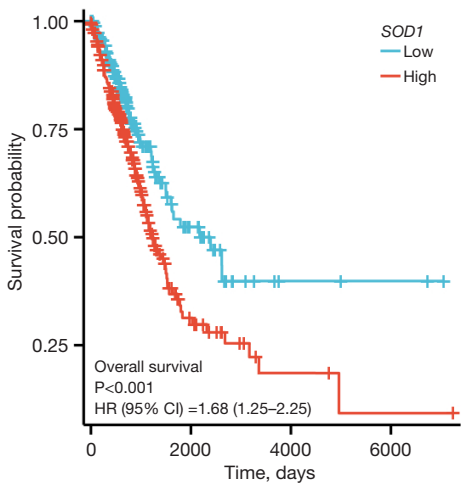

Figure 2 Kaplan-Meier survival curve for LUAD patients. The OS of all LUAD patients analyzed for *SOD1* expression. *SOD1*, copper-zinc superoxide dismutase; HR, hazard ratio; CI, confidence interval; LUAD, lung adenocarcinoma; OS, overall survival.

the polyketide metabolic process, the olefinic compound metabolic process, alcohol dehydrogenase ($NADP^+$) activity, aldo-keto reductase (NADP) activity, alditol ($NADP^+$) 1-oxidoreductase activity, and monocarboxylic acid binding. The KEGG pathway analysis identified the following four pathways that exhibited the strongest positive correlation with the expression of *SOD1*: folate biosynthesis, arachidonic acid metabolism, steroid hormone biosynthesis, and the metabolism of xenobiotics by cytochrome P450 (*Figure 4*). *Figure 5* shows the correlation analysis results between *SOD1* and all the other variables. Further, the study used the STRING database to create a network of the interactions between the proteins of the DEGs in LUAD. A STRING database analysis was also conducted to establish the PPI network (*Figure 6*).

GSEA investigation of SOD1

By conducting a GSEA of all the DEGs, numerous signaling pathways that were significantly enriched were identified, including the metabolism of xenobiotics by cytochrome P450, transsulfuration, one-carbon metabolism, and related pathways, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) modulation of host translation machinery, ribosomal RNA (rRNA) modification in the nucleus and cytosol, and glutathione metabolism (*Figure 7*).

Relationship between SOD1 expression and the immune cells that have infiltrated the tumor

TIMER was employed to examine the correlation between the immune cells that have infiltrated the tumor and *SOD1* expression (*Figure 8*). A positive correlation was observed between the levels of $CD4^+$ T cells (partial.cor =−0.195, $P=1.56\times10^{-5}$), macrophages (partial.cor =−0.182, $P=5.67\times10^{-5}$), neutrophils (partial.cor =−0.234, $P=1.93\times10^{-7}$), and dendritic cells (partial.cor =−0.155, $P=5.88\times10^{-4}$), and *SOD1* expression. We attempted to ascertain whether the

Table 3 Univariate and multivariate Cox regression analyses

Characteristics	Total, n	Univariate analysis		Multivariate analysis	
		HR (95% CI)	P value	HR (95% CI)	P value
SOD1	530				
Low	264	Reference		Reference	
High	266	1.677 (1.249–2.252)	<0.001*	1.589 (1.171–2.156)	0.003*
Gender	530				
Female	283	Reference			
Male	247	1.087 (0.816–1.448)	0.60		
Age	520				
≤65 years	257	Reference			
>65 years	263	1.216 (0.910–1.625)	0.20		
Race	472				
Asian	8	Reference			
Black or African American	55	1.911 (0.254–14.382)	0.53		
White	409	2.714 (0.380–19.403)	0.32		
Number pack years smoked	363				
<40	183	Reference			
≥40	180	1.073 (0.753–1.528)	0.70		
Pathologic stage	522				
Stage I	292	Reference		Reference	
Stage II	123	2.341 (1.638–3.346)	<0.001*	2.077 (1.417–3.043)	<0.001*
Stage III	81	3.576 (2.459–5.200)	<0.001*	3.337 (2.198–5.067)	<0.001*
Stage IV	26	3.819 (2.211–6.599)	<0.001*	2.943 (1.614–5.367)	<0.001*
Pathologic T stage	527				
T1	176	Reference		Reference	
T2	285	1.507 (1.059–2.146)	0.02*	1.247 (0.868–1.793)	0.23
T3	47	2.964 (1.762–4.986)	<0.001*	1.545 (0.877–2.722)	0.13
T4	19	3.357 (1.767–6.376)	<0.001*	1.287 (0.638–2.597)	0.50

*, P<0.05. HR, hazard ratio; CI, confidence interval; *SOD1*, copper-zinc superoxide dismutase.

immune microenvironment of the tumor differed between the LUAD patients who displayed high and low levels of *SOD1* expression. The 539 tumor samples were categorized into the high expression group (comprising 270 samples) and the low expression group (comprising 269 samples) based on the level of expression of *SOD1*. We assessed the levels of 24 subtypes of immune cells to assess the variation in their expression levels between the two groups with distinct expressions (*Figure 9*). NK cells, neutrophils, eosinophils, macrophages, NK CD56 bright cells, NK CD56 dim cells, Tgd cells, Tfh cells, Th2 cells, and Th17 cells were affected by *SOD1* expression.

The expression of SOD1 in lung cancer cells was validated using qPCR and Western blot techniques

In this study, we validated the expression of SOD1 in the normal bronchial epithelial cell line BEAS-2B and the

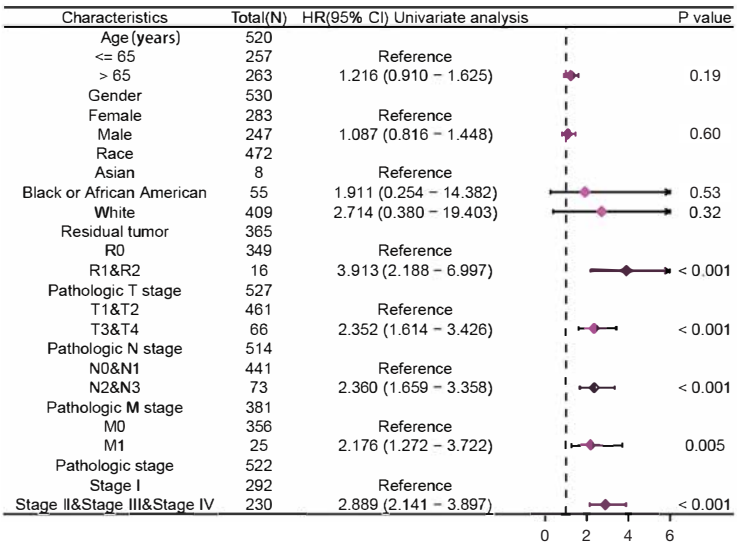

Figure 3 Forest plot for univariate Cox regression analysis of LUAD and *SOD1*-related functional enrichment analysis. HR, hazard ratio; CI, confidence interval; LUAD, lung adenocarcinoma; *SOD1*, copper-zinc superoxide dismutase.

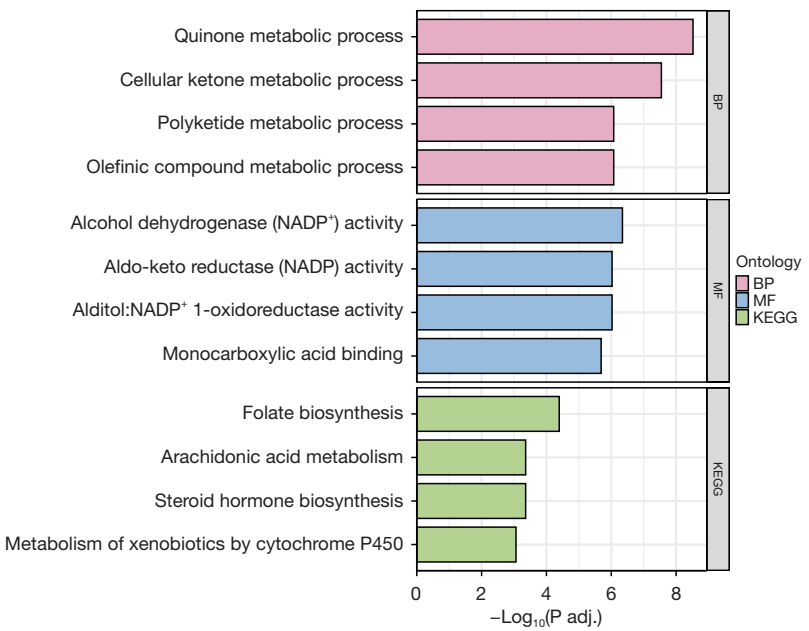

Figure 4 Enrichment of *SOD1* function in LUAD. BP, biological progress; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes; *SOD1*, copper-zinc superoxide dismutase; LUAD, lung adenocarcinoma.

human lung cancer cell lines NCI-H1395, NCI-H157, H1650, and Calu-3 using qPCR and Western blot techniques. The results showed that both the mRNA levels and protein expression levels of SOD1 were higher in the lung cancer cell lines than the normal bronchial epithelial cell line (*Figures 10,11*).

Discussion

LUAD is a heterogeneous disease at the molecular level. Histologically, biologically, and genetically, it is widely recognized that LUAD develops through multiple genetic and epigenetic changes, leading to diverse molecular profiles

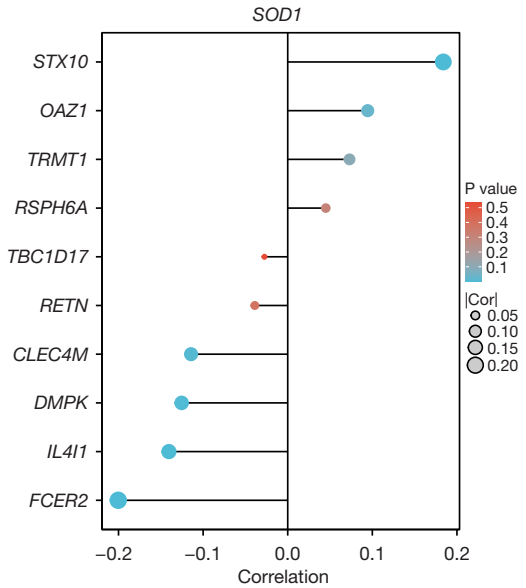

Figure 5 *SOD1* correlation lollipop chart. *SOD1*, copper-zinc superoxide dismutase.

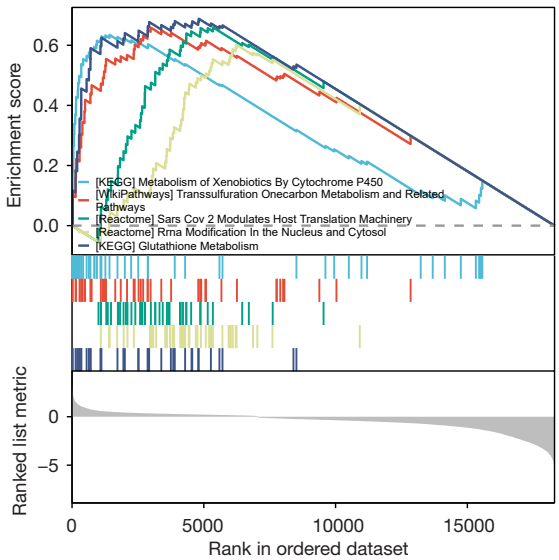

Figure 7 Enrichment plots from the GSEA. KEGG, Kyoto Encyclopedia of Genes and Genomes; GSEA, gene set enrichment analysis.

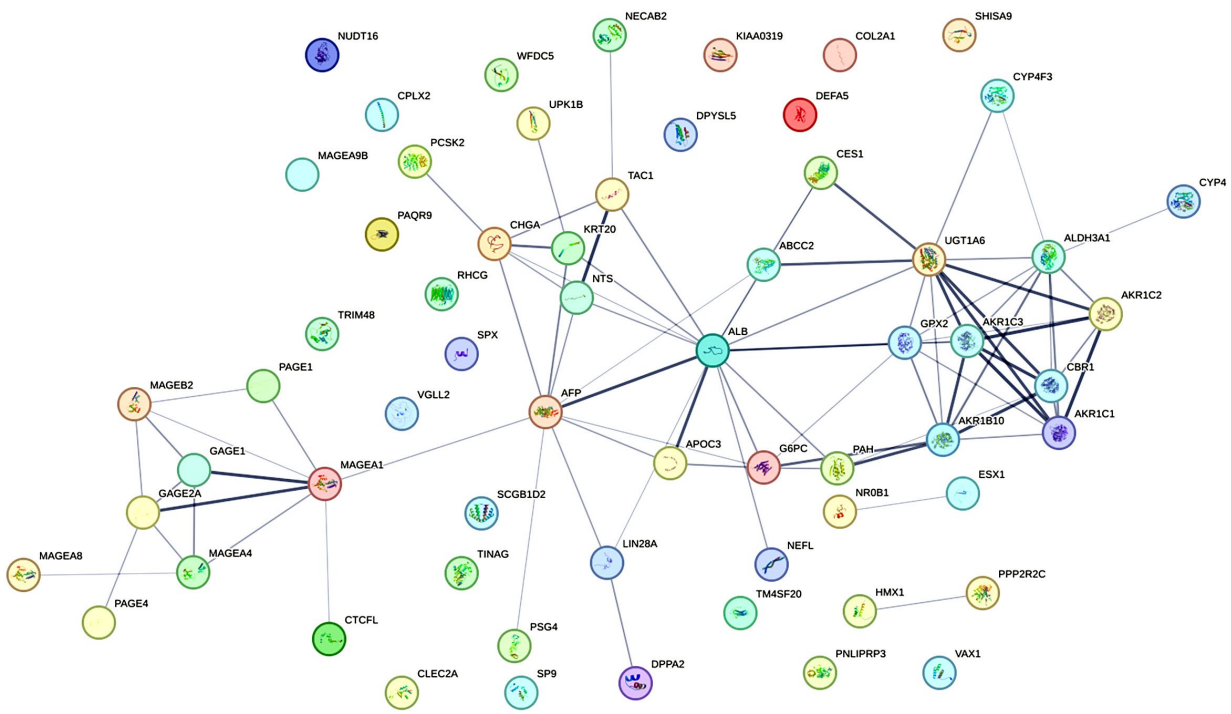

Figure 6 Analysis of the PPI network using STRING. PPI, protein-protein interaction; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins.

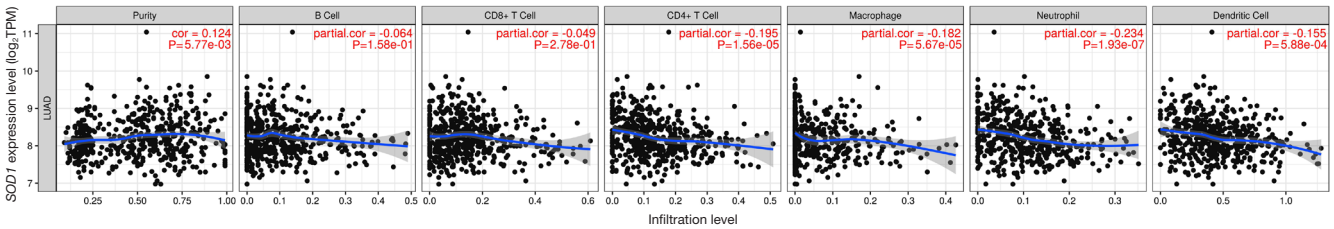

Figure 8 The correlations between levels of *SOD1* expression and immune infiltration. *SOD1*, copper-zinc superoxide dismutase; TPM, transcript per million; LUAD, lung adenocarcinoma.

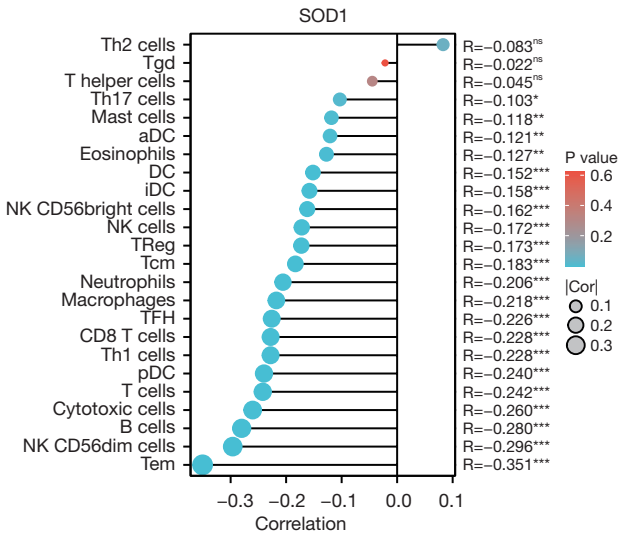

Figure 9 Proportions of immune cell subtypes in tumor samples with high and low expressions of *SOD1*. *, P<0.05; **, P<0.01; ***, P<0.001; ns, no significance. *SOD1*, copper-zinc disulfide sulfurase; Th, T helper; Tgd, gamma delta T; aDC, activated dendritic cell; DC, dendritic cell; iDC, immature dendritic cell; NK, natural killer; TReg, regulatory T cell; Tcm, central memory T cell; TFH, T follicular helper; CD, cluster of differentiation; pDC, plasmacytoid dendritic cell; Tem, effector memory T cell.

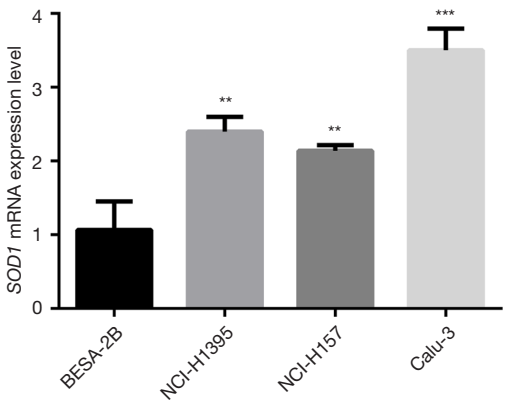

Figure 10 *SOD1* expression in LUAD cells. **, P<0.01; ***, P<0.001. *SOD1*, copper-zinc superoxide dismutase; mRNA, messenger RNA; LUAD, lung adenocarcinoma.

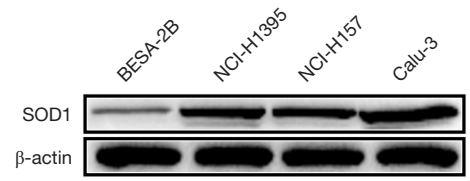

Figure 11 Detection of intracellular SOD1 protein expression levels by Western blot. SOD1, copper-zinc superoxide dismutase.

and clinical outcomes (12). Due to the absence of early clinical symptoms and effective screening methods, many patients already have metastases at the time of diagnosis (13). Therefore, novel mechanisms and therapeutic targets need to be discovered to address this concern.

The intermembrane space (IMS) is composed of the matrix, inner, and outer membranes of mitochondria, and the space between the inner and outer membranes (14). SOD1 is localized in the IMS, and the antioxidant mechanism of the IMS relies on the activity of SOD1. It has been suggested that the IMS portion of *SOD1* may also play a crucial role in the survival of cancer cells, as the overexpression of *SOD1* can promote the growth of cancer cells. A study has shown that SOD1 is overexpressed in a breast cancer cells (10), and the concentration of serum *SOD1* is positively correlated with the mortality rate of lung cancer.

In our investigation, we examined the potential of *SOD1* as a prognostic biomarker for LUAD. By conducting a bioinformatics analysis of TCGA database, we examined the expression profiles of the *SOD1* gene in matched normal and lung tissues from LUAD patients. Subsequently, we

conducted a Kaplan-Meier survival analysis and found that high *SOD1* expression was correlated with a poor prognosis in LUAD patients. Further, our univariate Cox analysis indicated a significant association between high *SOD1* expression and poor OS, while our multivariate Cox analysis further validated that the expression of the *SOD1* gene was an independent risk factor for OS among patients with LUAD. The GO term and KEGG pathway analyses performed in this study revealed that *SOD1* is related to the quinone metabolic process, cellular ketone metabolic process, polyketide metabolic process, olefinic compound metabolic process, alcohol dehydrogenase ($NADP^+$) activity, aldo-keto reductase (NADP) activity, alditol ($NADP^+$) 1-oxidoreductase activity, monocarboxylic acid binding, folate biosynthesis, arachidonic acid metabolism, steroid hormone biosynthesis, and the metabolism of xenobiotics by cytochrome P450. The synthesis of *SOD1* is associated with folate, which is involved in various metabolic processes such as nucleotide synthesis, DNA methylation, and cellular redox regulation. The inhibition of the folate pathway remains one of the most effective methods for treating multiple tumors. For instance, the commonly used folate-inhibiting drug, methotrexate is used to treat leukemia (15). Our study suggests that *SOD1* may provide a valid basis for the post-treatment of LAUD. Further, a study has indicated a possible association between high levels of folate and an increased risk of breast cancer in females (16). Additionally, other research has also shown that folate is involved in the pathology of epithelial ovarian cancer (17). In the metabolism of arachidonic acid, high concentrations of arachidonic acid in the ovarian cancer microenvironment are associated with poor clinical outcomes (18). In a study, a high level of arachidonic acid metabolism may serve as a favorable prognostic biomarker for breast cancer (19). Human cytochrome P450 enzymes play a crucial role in carcinogenesis by activating carcinogens and carcinogenic hormones. Our findings further support the association between *SOD1* and tumorigenesis. Among them, the upregulation of P450 1B1 promotes cancer cell proliferation and metastasis (20). The entry of polycyclic aromatic hydrocarbons and other substances into the lungs can lead to tumor formation by inducing DNA mutations through pathways involving cytochrome P450 enzymes (21). Among the superfamily of enzymes, such as cytochrome P450, CYP2U1 is closely associated with a poor prognosis and the pathological features of breast cancer, which suggests that *SOD1* may contribute to LUAD through a similar mechanism (22). A study has demonstrated that inhibition of SOD1 expression can induce cell cycle arrest at the G1 phase in NSCLC cells, including LUAD (23). This inhibition has also been shown to promote apoptosis in tumor cells. Previous research has identified other proteins with opposing functions. For instance, P16, acting as a tumor suppressor gene, interacts with cyclin-dependent kinases to induce cell cycle arrest at the G1 phase (24).

We conducted a further investigation into the relationship between *SOD1* and the level of immune infiltration. Our findings revealed a positive correlation between the expression levels of *SOD1* and $CD4^+$ T cells, macrophages, and dendritic cells. In previous studies, it has been shown that the goal of immunotherapy is to activate T lymphocytes for immune surveillance against cancer. Among these, CD4 T cells are associated with cancer immunity due to their auxiliary functions. $CD4^+$ T cells can kill tumors in a major histocompatibility complex (MHC) II-dependent manner (25). Similarly, CD4 T cells can eliminate established melanomas *in vivo* (26) and exhibit expanded CD4 cytotoxic T lymphocytes in various tumors such as lung cancer and colon cancer (27). Tumor-associated macrophages have the ability to promote tumor growth by producing a large amount of soluble mediators that support tumor cell proliferation, while also inhibiting anti-tumor immune responses (28). Conventionally, dendritic cells are considered a critical component of anti-tumor immunity, but their functional deficiencies may lead to immune suppression in cancer (29). A study has demonstrated that Reg3A can serve as a tumor-derived factor to promote dendritic cell growth, thereby contributing to the development of pancreatic cancer (30). Therefore, the effect of *SOD1* expression on tumors in the immune microenvironment remains unclear and requires further investigation.

Conclusions

In conclusion, the study showed that *SOD1* is significantly upregulated in LUAD and acts as an independent risk factor for OS in LUAD patients. The key pathways regulated by *SOD1* in LUAD include folate biosynthesis, arachidonic acid metabolism, the metabolism of xenobiotics by cytochrome P450, and other pathways that can affect cancer pathways. Moreover, the effect of *SOD1* on tumor-infiltrating immune cells suggests that it plays a crucial role in the development of LUAD. In our experiments, we were able to verify that the expression levels of *SOD1* were higher in tumor cells than normal cells in LUAD. Therefore,

SOD1 has the potential to act as both a diagnostic and prognostic marker for LUAD. However, this study still has certain limitations. We are currently collecting relevant pathological samples, and in future research, we will investigate the relationship between SOD1 and pathological samples from tumor patients.

Acknowledgments

Funding: This work was supported by the Chuxiong Prefecture People's Hospital Scientific Research Fund (No. 2021J21 to L.G.).

Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1400/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1400/dss

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1400/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-1400/coif). D.K. serves as an unpaid editorial board member of *Translational Cancer Research* from August 2023 to July 2025. L.G. reports funding from the Chuxiong Prefecture People's Hospital Scientific Research Fund (No. 2021J21). The other authors have no conflicts of interest to declare.

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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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(English Language Editor: L. Huleatt)

Cite this article as: Gao L, Wang W, Ma H, Yin M, Yang X, Han R, Ohara S, Kim D, Wang G. Bioinformatics analysis reveals *SOD1* is a prognostic factor in lung adenocarcinoma. Transl Cancer Res 2024;13(10):5522-5534. doi: 10.21037/tcr-24-1400