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ROLE OF COPPER DEATH MARKERS ATP7A/ATP7B IN REGULATING ANTIOXIDANTS GSH AND SOD IN THE PATHOGENESIS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

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Abstract: Objective: To investigate the underlying mechanisms by which cuproptosis-related markers ATP7A/ATP7B, together with the antioxidant regulators GSH and SOD, contribute to the pathogenesis of COPD. Methods: A retrospective study was conducted using clinical data from 102 patients diagnosed with COPD and enrolled between March 2021 and December 2024, who constituted the study population.Sixty Wholesome subjects receiving medical screenings in the identical timeframe were chosen as the reference group. Multiplex concurrent fluorimetric signal quantitative PCR and WB were used to detect ATP7A, ATP7B, SLC31A1, FDX1, and DLAT in all participants. Baseline data, routine laboratory tests, COPD assessment test (CAT) scores, and pulmonary function indicators were also collected. Results: In the study group, ATP7A, ATP7B, FDX1, and DLAT expression levels were significantly lower, while SLC31A1 was considerably elevated relative to the reference group ($P<0.05$). No notable discrepancies were found in sex or alcohol history. However, the study group had significantly higher age, rates of diabetes and hypertension, smoking history, CRP, LTB4, ICAM-1, TNF-α, MDA, CAT scores, and airway resistance (Raw), and significantly lower GSH, SOD, FEV1, FVC, and PEFR levels ($P<0.05$). All these factors, including the gene expressions and clinical indicators, were significantly correlated with COPD ($P<0.05$). ROC analysis showed that age, CRP, IL-6, IL-8, IL-10, IL-17, LTB4, ICAM-1, TNF-α, MDA, CAT, and Raw had strong predictive value (AUC > 0.8), with a combined diagnostic AUC of 1. Conclusion: ATP7A/ATP7B, GSH, and SOD may reduce cuproptosis and inhibit the onset and progression of COPD. However, their individual predictive value is limited, and other indicators should be combined in clinical diagnosis.
Keywords: ATP7A; ATP7B; GSH; SOD; COPD

1 INTRODUCTION

As an essential trace element, copper participates in enzymatic reactions as $Cu^{+}$and Cu^{2+}[1]. it is stored in the liver, distributed to tissues, or excreted via ATP7A/ATP7B transporters[2–3]. Dysregulated copper transport causes intracellular accumulation, disrupting the tricarboxylic acid cycle and mitochondrial function to induce cuproptosis[4], which is followed by oxidative stress, inflammation, and organ dysfunction[5]. ATP7A/ATP7B are cuproptosis biomarkers, and COPD is a common respiratory disorder marked by airflow limitation and persistent inflammation [6].
Animal studies show elevated copper metabolism-related genes and transporters in COPD[7], with copper accumulating in alveolar cells of smoking patients due to impaired macrophage function[8]. However, clinical studies on ATP7A/ATP7B in COPD are limited, and their underlying mechanisms remain unclear[9]. GSH chelates free Cu^{+}, while SOD scavenges free radicals[10], and serves as a potential therapeutic target [11]. This study explores their roles in COPD to identify new therapeutic targets.

2 MATERIALS AND METHODS

2.1 Source of Cases

A lookback investigation was performed regarding the clinical data of 102 patients with COPD from March 2021 to December 2024, who were assigned to the study group. Among them, 37 cases were in the acute exacerbation phase of COPD (AECOPD), and 65 cases were in the stable phase of COPD (SCOPD). Additionally, 60 healthy subjects undertaking physical appraisals during the same interval were picked as the control cohort.
(1) Inclusion criteria:
Participants were aged ≥18 years and had executed informed assent forms; COPD diagnosis in the study group was confirmed via chest CT, serological tests, bronchoalveolar lavage, and secretion cultures;
All individuals in the control group had normal function of major organs;
No history of pulmonary or respiratory tract surgery in any participant;
The ethics committee gave its sanction to the study protocol.
(2) Exclusion criteria:

Presence of tuberculosis, malignancies, hematologic disorders, severe psychiatric conditions, neurological dysfunctions, or cardiovascular and cerebrovascular diseases;
Systemic infectious diseases, chest fractures, or other contagious diseases;
Chest trauma, gastrointestinal bleeding, or other active bleeding disorders;
Deficient clinical datasets.

2.2 Methods

2.2.1 Materials source

The human copper ion transporter ATP7A assay kit used was procured from Shanghai Ke Chengwei Biotechnology Co., Ltd., possessing a concentration of 1 mg/mL and 100 μL volume. Human copper ion transporter ATP7B assay reagent set was obtained from Wenzhou Kemiao Biotechnology, also with a concentration of 1 mg/mL and 100 μL volume. The reduced glutathione assay kit used herein was sourced from Shanghai YuanYe Biotechnology, with a purity of 99% and a specification of 5 g. The SOD detection set was acquired from Qiyi Biotech Co., Ltd., with a purity of 99%, and provided in 50 tubes for 48 samples.The human high-affinity copper uptake protein SLC31A1 detection kit was sourced from Wuhan Jingxi Biotechnology, with a concentration of 1 mg/mL and two available specifications of 48-well and 96-well plates. The human ferredoxin 1 detection kit was obtained from Shanghai Youkewei Biotechnology Co., Ltd.; it had a purity of more than 90% and was also provided in 48-well and 96-well plate formats.
Recombinant dihydrolipoyl transacetylase protein was purchased from Shanghai Xinyu Biotechnology, with a purity of over 90% and a specification of 100 μg. The multiplex real-time fluorescent quantitative PCR kit was procured from Shanghai Yisheng Biotechnology Co., Ltd., with a specification of 100 tests per kit. Trizol reagent was purchased from Shanghai Xiyan Scientific Instruments Co., Ltd., with an elution volume of 20–600 μL and a specification of 100 mL. The BCA protein concentration assay kit was sourced from Shanghai Lusen Bioengineering Co., Ltd., with a specification of RC250T. Xanthine oxidase and other essential reagents, cell culture plates and flasks, cell counting plates, centrifuge tubes, pipettes, and pipette tips were all obtained from Shanghai Fusheng Industrial Co., Ltd. Phosphate-buffered saline (PBS) was self-prepared using the following formulation: 8 grams of NaCl, 0.2 grams of KCl, 1.44, and 800 mL of deionized water.

2.2.2 Main instruments

The high-speed refrigerated benchtop centrifuge TGL-20M was purchased from Hunan Hukang Centrifuge Co., Ltd., registration number Xiang Chang Xie Bei Zi 20170027. The low-speed benchtop centrifuge LTA-1600 was obtained from Zhuhai Langtai Biotechnology Co., Ltd., registration number Yue Zhu Xie Bei 20210060. The fully automatic cell analyzer DX-80 was purchased from Jiangsu Jingjiang Diagnostic Technology Co., Ltd., registration number Su Xie Zhu Zhun 20222222142. The biological microscope BM2100 was acquired from Nanjing Jiangnan Yongxin Optical Co., Ltd., registration number Su Xie Zhu Zhun 20172221144. The multifunctional microplate reader Multiskan FC Thermo was purchased from Jinan Laibao Medical Equipment Co., Ltd., registration number Hu Xie Zhu Zhun 20182400073. The UV-visible spectrophotometer L6S was also obtained from Jinan Laibao Medical Equipment Co., Ltd., registration number Lu Ji Yao Jian Xie Jing Ying Bei 20170800. The FGY-200 pulmonary function testing device was purchased from Wuhan Kangbeinuo Medical Equipment Co., Ltd., registration number Wan Xie Zhu Zhun 20162070290.

2.2.3 qPCR detection

A 10 mL peripheral blood sample was obtained from each participant and submit to spinning at a spin rate of 3,000 rpm over 10 min with a rotor radius of 10 cm. Following centrifugation, the resulting supernatant and cellular pellet were carefully isolated and kept at −80 °C until further processing. Prior to RNA extraction, the precipitate was first thawed at −4 °C and then at 0 °C. Subsequently, 1.0–1.5 mL of the rehomogenized precipitate was conveyed to a small-volume centrifuge tube. For RNA isolation, 1 volume of TRIzol reagent was added per 1×10^5–1×10^6 cells, followed by thorough mixing. After incubation at room temperature for 5 min, 200 μL of chloroform was added and the mixture was vortexed briefly, then centrifuged at 12,000×g for 15 min at 4 °C. The aqueous phase was carefully recovered and mixed with an equal volume of isopropanol. Incubate the solution at room temperature for 10 minutes, then centrifuge at 12,000×g for 10 minutes at 4 °C. Collect the resulting RNA precipitate, wash it with 1 mL of 75% ethanol, and centrifuge again at 7,500×g for 5 minutes. After the precipitate is air-dried naturally, add 30 μL of RNase-free water to dissolve it, then quantify the RNA concentration and adjusted to a final concentration of 200 ng/μL. RNA purity is evaluated by measuring the A260/A280 absorbance ratio, with an acceptable range of 1.8 to 2.0. All samples meeting this purity standard are preserved at −80 °C for subsequent experimental analysis.For qPCR, cDNA was generated from 1 microgram of total RNA utilizing a miRNA RT reagent set, with ACTB as the internal reference gene. Each qPCR reaction had a final reaction volume of 10 μL, incorporating 6 μL of nuclease-free water, 2 μL of cDNA scaffold, and 1 microliter per piece of the sense and antisense primers.Relative miRNA expression levels were quantified via the 2^-ΔΔCt approach, under amplification settings of 95°C over 5 minutes and subsequently 40 cycles of 94°C over 20 seconds, 62°C over 30s, alongside 70°C over 20s.The sequences of the primers are documented in Supplementary Table 1.

Table 1 Primer Sequences

Primer name		Primer sequence
ATP7A	Upstream primer	5’-AGGTCTCTAAGGCCACACCGG-3’

ATP7B	downstream primer	5’-GGACCCTTCGGTTATCCGTAA-3’
	Upstream primer	5’-AATCCGGTCGGAATCGTATCCA-3’
	downstream primer	5’-GTTGTCCGGTATTAACGGATATTGA-3’
SLC31A1	Upstream primer	5’-GGAATGGGGATCCTTACTTACCA-3’
	downstream primer	5’-AATCGGGTTCTGATAGTAATTGTAT-3’
FDX1	Upstream primer	5’-GGTTAATGAGGCCTAACCATAAC-3’
	downstream primer	5’-AATTCGGGTACGGGTACTAGCACCT-3’
DLAT	Upstream primer	5’-TTTCTGGTATATAAGGTACCCATAC-3’
	downstream primer	5’-CCTAAGTATTCCGATCCTTATTAGTG-3’

2.2.4 Western Blot (WB) detection of proteins

(1) Preparation: Based on the number of standards and samples, prepare the BCA working solution via blending BCA reagent with Cu reagent at a rate of 50:1. Mix thoroughly, and store at room temperature. The working solution remains stable for use within 24 hours. (2) Detection: After routine thawing, an appropriate amount of RIPA lysis buffer was supplemented to the serum samples, followed by ultrasonic disruption. The lysates were spun down at 12,000 ×g for 10 minutes under 4°C eliminate cellular debris.The clarified supernatant was pipetted into a new centrifuge tube, and the concentration of protein was measured with the BCA assay. A 10 μL aliquot of bovine serum albumin (BSA) standard was diluted with the provided PBS to a final volume of 100 μL(0.5 mg/mL). Then, 0 μL, 5μL, 10μL, 15μL, and 20μL of the standard solution were pipetted into the wells of the enzyme-linked plate, with PBS supplemented to make the volume of each well to 20 μL. For protein samples, 5 μL was added per well and PBS was introduced to adjust the final volume to 20 μL.Subsequently, 200 μL of BCA working solution was dispensed into each well, and the culture plate was maintained at 37 °C for 15–30 min. The absorbance at 562 nm was measured using a microplate reader, and the protein concentration was calculated according to the standard curve and sample volume.

2.3 Data Collection

Baseline data: It included age, gender, disease duration of chronic obstructive pulmonary disease (COPD), and comorbidity status.

Routine Laboratory tests:The ELISA was used to detect the expression levels of GSH, CRP, interleukin-6, interleukin-8, interleukin-10, interleukin-17, leukotriene B4, ICAM-1, and tumor necrosis factor-αin serum. The level of SOD was determined by the WST-8 colorimetric method, while the serum MDA level was quantitatively detected via the TBARS assay.

The Chronic Obstructive Pulmonary Disease Assessment Test was employed to evaluate the severity of patients' symptoms and the impact of the disease on their daily activities. This scale consists of a total of 8 assessment items, specifically covering aspects such as cough, expectoration, chest tightness, difficulty in climbing stairs or walking uphill, limitations in daily activities, inconvenience in going out, poor sleep quality, and mental health status.

Each question was rated on a 0-to-5 scale; an overall score of less than 10 indicated a mild impact of the disease on daily life, and a higher total score reflected a greater disease burden.

Pulmonary function was assessed by measuring pulmonary function indices, including FEV1, FVC, airway resistance (Raw) and PEFR.

2.4 Statistical Analysis

SPSS 27.0 software was utilized for data collation and evaluation; categorical data were depicted as ‘n(%)’ (independent samples Chi-square test for intergroup comparisons), continuous data as the mean and standard deviation (x±s), Pearson correlation analysis explored associations between indicators and COPD, ROC curve and AUC appraised predictive efficacy, and significance was considered as deemed at $P < 0.05$.

3 RESULTS

3.1 To Assess the Expression of the Target Genes

Figs. 1A and 1B exhibited faint RNA bands of ATP7A/ATP7B (low concentration); Fig. 1C displayed fragmented RNA bands of SLC31A1 (low concentration). Conversely, the RNA bands of FDX1 and DLAT (Figs. 1D and 1E) were distinct and intact (high concentration, minimal degradation, *P<0.05, **P<0.01).

The protein bands of ATP7A/ATP7B attenuated (increased degradation, diminished expression); the protein bands of SLC31A1 broadened slightly yet remained the narrowest. The protein bands of FDX1 were the broadest and most intense, followed by DLAT (both intact, high expression, Fig. 2, *P<0.05, **P<0.01).

The expression and fluorescence intensity of ATP7A/ATP7B diminished progressively (Figs. 3A and 3B); the fluorescence of SLC31A1 was faint (Fig. 3C). The expression of FDX1 and DLAT elevated gradually, among which

FDX1 exhibited the strongest fluorescence intensity (Figs. 3D and 3E, *P<0.05, **P<0.01). Blue = nucleus, green = protein levels.

3.2 Comparison of Copper Death-Related Gene Expression Between the Two Groups

As shown in Supplementary Table 2, the abundance of ATP7A, ATP7B, FDX1, and DLAT genes in the study group were obviously lower than those in the control group, whereas the expression content of SLC31A1 was notably high in contrast to those in the control group.

Table 2 Comparison of Cuproptosis-Related Gene Expression Between the Two Groups ($\bar{x}$ ±s)

Item	Research Group (n=102)	Control group (n=60)	*t*	*P*
ATP7A	1.11±0.31	1.47±0.43	6.164	<0.001
ATP7B	1.07±0.22	1.39±0.35	7.147	<0.001
SLC31A1	1.03±0.47	0.86±0.34	2.449	0.015
FDX1	1.45±0.41	1.86±0.65	4.924	<0.001
DLAT	1.38±0.46	1.93±0.57	6.716	<0.001

3.3 Comparison of Cuproptosis-Related Gene Expression in Different Stages of COPD in the Study Group

In the study cohort, the expression profiles of *ATP7A*, *ATP7B*, *FDX1*, and *DLAT* were significantly reduced in the acute exacerbation COPD (AECOPD) subgroup compared to the stable COPD (SCOPD) subgroup. By contrast, no statistically marked variation existed in the expression of *SLC31A1* in the two subgroups, as shown in Supplementary Table 3.

Table 3 Comparison of Cuproptosis-Related Gene Expression in Different Stages of COPD in the Study Group ($\bar{x}$±s)

Item	AECOPD Group (n=37)	SCOPD Group (n=65)	*t*	*P*
ATP7A	0.96±0.31	1.19±0.27	3.918	<0.001
ATP7B	0.97±0.22	1.13±0.19	3.859	<0.001
SLC31A1	0.99±0.31	1.02±0.47	0.347	0.729
FDX1	1.24±0.32	1.56±0.40	4.164	<0.001
DLAT	1.10±0.42	1.53±0.41	5.048	<0.001

3.4 Comparison of Clinical Characteristics

No notable variations in gender were found between the two cohorts and alcohol history. However, the study cohort displayed significantly higher levels of age, diabetes, hypertension, smoking history, CRP, IL-6, IL-8, IL-10, LTB4, ICAM-1, TNF-α, MDA, CAT scores,airway resistance (Raw), while levels of GSH, SOD, FEV1, FVC, and PEFR were significantly lower in comparison with the control group. All differences reached statistical relevance (P < 0.05), as illustrated in Supplementary Table 4.

Table 4 Comparison of Clinical Characteristics Between the Two Groups ($\bar{x}$ ±s, n)

Item		Research Group (n=102)	Control group (n=60)	*t/*	*P*
Sex	Male	61	39	0.432	0.511
	Female	41	21		
Age (years)		64.21±7.83	53.45±5.18	9.487	<0.001
Diabetes	Yes	17	0	11.172	0.001
	No	85	60		
Hypertension	Yes	12	0	7.624	0.006
	No	90	60		
Smoking history	Yes	57	11	21.869	<0.001
	No	45	49		
Drinking history	Yes	24	9	1.694	0.193
	No	78	51		
GSH (U/L)		46.17±7.28	62.35±6.49	14.209	<0.001
CRP (mg/L)		27.30±8.05	9.52±4.37	15.782	<0.001
IL-6 (ng/L)		30.25±5.43	11.37±3.50	24.129	<0.001

IL-8 (ng/L)	24.10±6.27	10.55±2.16	16.167	<0.001
IL-10 (ng/L)	17.35±4.26	8.73±2.38	14.396	<0.001
IL-17 (ng/L)	22.36±5.49	13.46±3.40	11.335	<0.001
LTB4 (ng/mL)	25.73±6.34	11.81±2.19	16.422	<0.001
ICAM-1 (μg/L)	180.65±39.26	62.25±7.45	23.089	<0.001
TNF-α (ng/L)	27.03±6.45	11.25±2.89	17.905	<0.001
SOD (U/L)	77.48±8.36	115.65±10.47	25.516	<0.001
MDA (mmol/L)	4.79±1.14	2.07±0.85	16.037	<0.001
CAT (score)	16.78±4.32	7.54±1.69	15.853	<0.001
FEV1 (L)	1.81±0.34	2.17±0.27	7.002	<0.001
FVC (L)	1.90±0.37	2.34±0.45	6.738	<0.001
Raw (%)	64.27±6.12	55.78±4.39	9.410	<0.001
PEFR (L/min)	4.82±1.58	5.36±1.24	2.267	0.025

3.5 Comparison of Clinical Characteristics in Different Stages of COPD in the Study Group

Within the study cohort, no marked variations were observed between the AECOPD and SCOPD subgroups regarding gender, age, diabetes, hypertension, smoking and alcohol history, CRP, IL-8, IL-10, IL-17, LTB4, ICAM-1, CAT score, FEV1, FVC, Raw, and PEFR (P>0.05). However, GSH and SOD levels were significantly lower in the AECOPD group, while IL-6 and MDA levels were significantly higher compared to the SCOPD group (P < 0.05), as shown in Supplementary Table 5.

Table 5 Comparison of Clinical Characteristics in Different Stages of COPD in the Study Group ($\bar{x}$ ±s, n)

Item		AECOPD Group (n=37)	SCOPD Group (n=65)	*t*	*P*
Sex	Male	25	36	1.456	0.228
	Female	12	29		
Age (years)		65.59±6.82	63.41±8.30	1.357	0.178
Diabetes	Yes	9	8	2.451	0.117
	No	28	57		
Hypertension	Yes	2	10	2.262	0.133
	No	35	55		
Smoking history	Yes	14	31	0.929	0.335
	No	23	34		
Drinking history	Yes	8	16	0.117	0.732
	No	29	49		
GSH (U/L)		44.03±6.57	47.38±7.44	2.279	0.025
CRP (mg/L)		26.97±6.92	27.48±8.68	0.306	0.760
IL-6 (ng/L)		31.69±5.32	29.42±5.35	2.065	0.042
IL-8 (ng/L)		22.66±5.50	24.91±6.56	1.762	0.081
IL-10 (ng/L)		16.60±4.08	17.77±4.34	1.337	0.184
IL-17 (ng/L)		21.70±5.47	22.73±5.50	0.911	0.364
LTB4 (ng/mL)		26.02±5.26	25.56±6.92	0.351	0.727

ICAM-1 (μg/L)	180.03±38.54	180.99±39.96	0.118	0.906
TNF-α (ng/L)	27.12±6.24	26.97±6.61	0.114	0.910
SOD (U/L)	75.21±9.75	78.77±7.23	2.101	0.038
MDA (mmol/L)	5.30±0.83	4.51±1.19	3.571	0.001
CAT (score)	16.93±4.94	16.68±3.97	0.285	0.776
FEV1 (L)	1.77±0.35	1.83±0.33	0.864	0.390
FVC (L)	1.92±0.39	1.88±0.35	0.532	0.596
Raw (%)	63.12±5.73	64.92±6.29	1.434	0.155
PEFR (L/min)	4.97±1.84	4.72±1.41	0.769	0.444

3.6 Correlation Analysis of Each Indicator with COPD

ATP7A, ATP7B, SLC31A1, FDX1, DLAT, Age, Diabetes, Hypertension, Smoking history, GSH, CRP, IL-6, IL-8, IL-10, IL-17, LTB4, ICAM-1, SOD, MDA, CAT, FEV1, FVC, Raw, and PEFR all showed significant correlations with COPD (P<0.05), as illustrated in Supplementary Table 6.

Table 6 Correlation Analysis of Each Indicator with COPD

Variable	*r*	*P*
ATP7A	-0.421	<0.001
ATP7B	-0.467	<0.001
SLC31A1	0.160	0.009
FDX1	-0.345	<0.001
DLAT	-0.437	<0.001
Age	0.527	<0.001
Diabetes	0.209	<0.001
Hypertension	0.171	0.005
Smoking history	0.315	<0.001
GSH	-0.692	<0.001
CRP	0.712	<0.001
IL-6	0.844	<0.001
IL-8	0.714	<0.001
IL-10	0.680	<0.001
IL-17	0.593	<0.001
LTB4	0.719	<0.001
ICAM-1	0.821	<0.001
TNF-α	0.752	<0.001
SOD	-0.875	<0.001
MDA	0.730	<0.001
CAT	0.708	<0.001
FEV1	-0.426	<0.001
FVC	-0.438	<0.001
Raw	0.572	<0.001
PEFR	-0.150	0.015

3.7 ROC Curve Analysis of Each Indicator for Predicting COPD

According to the ROC curve analysis, the prognostic capacity of Age, CRP, IL-6, IL-8, IL-10, LTB4, ICAM-1, MDA, CAT, and Raw for COPD was high (AUC>0.8). The combined diagnostic AUC of these indicators was 1, as shown in Figure 4 and Supplementary Table 7.

Table 7 Sensitivity and Specificity of Various Indicators in Predicting COPD

Variable	AUC	95%IC	sensitivity(%)	specificity(%)	*P*
ATP7A	0.241	0.162~0.320	98.00	1.70	<0.001
ATP7B	0.223	0.149~0.298	98.00	1.70	<0.001
SLC31A1	0.612	0.540~0.684	52.00	76.70	0.008
FDX1	0.283	0.198~0.368	97.10	8.30	<0.001
DLAT	0.217	0.145~0.289	98.00	1.70	<0.001
Age	0.881	0.840~0.921	81.40	83.30	<0.001
Diabetes	0.583	0.508~0.659	16.70	100.00	0.050
Hypertension	0.559	0.481~0.637	11.80	100.00	0.166
Smoking history	0.688	0.615~0.760	55.90	81.70	<0.001
GSH	0.041	0.017~0.064	100.00	0	<0.001
CRP	0.970	0.953~0.988	91.20	98.30	<0.001

IL-6	0.998	0.994~1.000	99.00	96.70	<0.001
IL-8	0.975	0.958~0.993	93.10	98.30	<0.001
IL-10	0.954	0.931~0.977	89.20	93.30	<0.001
IL-17	0.918	0.886~0.951	75.50	96.70	<0.001
LTB4	0.966	0.944~0.988	92.20	100.00	<0.001
ICAM-1	1.000	1.000	100.00	98.30	<0.001
TNF-α	0.989	0.980~0.998	97.10	95.00	<0.001
SOD	0.002	0.000~0.006	100.00	0	<0.001
MDA	0.970	0.952~0.987	86.30	96.70	<0.001
CAT	0.990	0.982~0.998	94.10	98.30	<0.001
FEV1	0.211	0.152~0.269	100.00	0	<0.001
FVC	0.212	0.144~0.280	98.00	1.70	<0.001
Raw	0.882	0.840~0.924	75.50	95.00	<0.001
PEFR	0.388	0.312~0.464	2.90	100.00	0.009
Combined diagnosis	1.000	1.000	1.000	0	<0.001

4 DISCUSSION

Copper death is a unique form of cell death primarily caused by the imbalance of Cu homeostasis both inside and outside the cell. This process involves multiple metabolic pathways and is closely linked to organismal function [12]. In terms of clinical evaluation, the Chronic Obstructive Pulmonary Disease Assessment Test (CAT) was used to assess the severity of patients' symptoms and the disease's interference with daily lives. This 8-item scale covers cough, expectoration, daily activity restrictions, going-out inconvenience, poor sleep quality, and mental health conditions.As a key element in cell signaling and mitochondrial function regulation, copper homeostasis is mainly maintained by copper transport proteins SLC31A1, ATP7A, and ATP7B [13-14].

SLC31A1, located on the cell membrane, mediates copper uptake; its overexpression increases intracellular $Cu^{+}$and decreases extracellular free Cu^{+}. ATP7A and ATP7B are responsible for copper efflux; reduced expression hinders excess Cu^{+}excretion and induces Cu^{+}accumulation in the brain and liver, leading to central nervous system diseases and abnormal cell proliferation. Excessive Cu^{+}disrupts metabolic pathways, the electron transport chain and tricarboxylic acid cycle, promoting diseases like cancer, Menkes disease, Wilson disease, and Alzheimer's disease.This study found significant differences in copper metabolism-related gene expression between the study and control groups[15-16].

ATP7A, ATP7B, FDX1, and DLAT were lower, while SLC31A1 was higher in the study group. This is attributed to COPD's key pathological mechanisms [17]. which reduce copper transport efficiency of ATP7A, ATP7B, and SLC31A1, leading to increased Cu^{+}absorption, excessive free radicals, and severe lung function impairment.FDX1 and DLAT also regulate copper-mediated cellular processes[18-20]. As an upstream regulator of protein acetylation with stable structure and electron transfer capacity, FDX1 activates cytochrome P450 enzymes, participates in DLAT lipoic acid modification, and reduces $Cu^{2+}$to more toxic Cu^{+}. DLAT lipoic acid modification triggers heteropolymerization, enhancing Cu^{+}-mediated cytotoxicity and tissue oxygen consumption. Within the study group, AECOPD patients had lower ATP7A, ATP7B, FDX1, and DLAT expression than SCOPD patients, consistent with Zhao R et al.'s finding of differential Cu^{+} metabolism-related gene expression between normal and diseased tissues [21].

These results suggest ATP7A, ATP7B, SLC31A1, FDX1, and DLAT are potential regulatory targets for copper-related diseases[22-23], which may inhibit oxidative stress, improve the cellular microenvironment, optimize immunotherapy, slow tumor progression, and improve COPD prognosis. ATP7A and ATP7B activity is regulated by intracellular Cu^{+}levels and Cu^{+}-binding proteins[24]. Cu^{+}transport relies on ATP7A and ATP7B, which provide mitochondrial energy, promote extracellular matrix remodeling, and maintain liver hematopoietic function[25]. Therefore, to use ATP7A and ATP7B as new therapeutic targets for COPD or other copper death-related diseases, it is crucial to closely monitor Cu+ levels in different tissues and organs to avoid excessive depletion of endogenous copper, which could lead to further reductions in ATP7A and ATP7B. Clinical characteristics and correlation analysis reveal that COPD is primarily associated with ATP7A, ATP7B, SLC31A1, FDX1, DLAT, age, diabetes, hypertension, smoking history, inflammatory factors, and lung function, which aligns with most studies [26-27]. Huo S et al. reported that advanced glycosylation end products (AGEs)[28], induced by sustained hyperglycemia, exacerbate intracellular Cu+ accumulation and interact with copper compounds and the copper ion carrier Elesclomol-Cu (ES-Cu) in the heart. At the same time, AGEs can upregulate the signaling pathways of SLC31A1 and FDX1, disrupting copper homeostasis[29].

This would lead to myocardial cell death, causing depletion of GSH, lipid peroxidation, and further accelerating inflammatory damage to the vascular endothelium, which in turn increases the risk of various cardiovascular diseases[30]. Investigations into AML cell proliferation and copper-driven cell death have uncovered critical links between ES-Cu exposure and intracellular homeostasis disruption[31]. Specifically, escalating ES-Cu concentrations correlate with a marked reduction in intracellular GSH and diminished expression of ATP7B, FDX1, and DLAT proteins—findings corroborated by WB analysis. Targeting the ES-Cu pathway, therefore, presents a viable strategy to modulate copper-mediated cytotoxicity and reinstate equilibrium among copper metabolism-regulating proteins.

GSH functions as a key antioxidant peptide, tasked with mitigating intracellular Cu^{+}excess. Impaired GSH synthesis or its overconsumption triggers dual pathological consequences: intracellular Cu^{+}accumulation and robust ferrous ion aggregation, both of which collectively drive a substantial surge in lipid peroxidation byproducts[32-33]. In the AECOPD,SOD and GSH were markedly lower than in the SCOPD sample, while IL-6 and MDA levels were

significantly higher. This is because[34-36]: (1) GSH can chelate intracellular Cu+ and also act as a Cu+ carrier, transporting it to metallothionein, thereby reducing oxidative stress within cells. (2) SOD effectively scavenges free radicals, reduces the production of oxidants, and alleviates or stabilizes COPD. (3) With the reduction of oxidative stress and free radicals, harmful substances and inflammatory factors produced during the oxidative stress process, such as MDA and IL-6, are also decreased. Additionally, SOD, GSH, and MDA are commonly used clinical antioxidants, which are of high value in assessing the effectiveness of COPD treatment and acute exacerbation[37].

5 CONCLUSION

ATP7A/ATP7B, GSH, and SOD are significantly correlated with the development of COPD, and these indicators can be used as therapeutic targets to control the progression of the disease by reducing copper-induced cell death. However, the predictive value of ATP7A/ATP7B, GSH, and SOD is relatively poor. Additionally, as there are many factors contributing to COPD and copper-induced cell death, optimizing clinical efficacy and selecting treatment targets for this disease should be cautiously determined by combining clinical indicators and making flexible judgments.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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INFORMED CONSENT STATEMENT

All study procedures were approved by the Ethics Committee of Hunan Provincial People’s Hospital, the First Affiliated Hospital of Hunan Normal University (No. 2023124).

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