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ORIGINAL RESEARCH

Cuproptosis-Related Gene – *SLC31A1, FDX1* and *ATP7B* – Polymorphisms are Associated with Risk of Lung Cancer

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Background: Cuproptosis is a novel copper-dependent cell death, and the copper level was increased in lung cancer patients. However, few studies evaluated the association between single-nucleotide polymorphisms (SNPs) in cuproptosis-related genes and lung cancer risk.

Methods: Six SNPs of the *SLC31A1, FDX1* and *ATP7B* genes were genotyped in a case–control cohort including 650 lung cancer cases and 650 controls using the MassARRAY platform.

Results: The minor alleles of *SLC31A1*-rs10981694 and *FDX1*-rs10488764 were associated with an increased risk of lung cancer (rs10981694: OR=1.455, 95% CI: 1.201–1.763, $p<0.001$; rs10488764: OR=1.483, 95% CI: 1.244–1.768, $p<0.001$). In contrast, the minor alleles of rs9535826 and rs9535828 in *ATP7B* were related to a decreased risk of the disease (rs9535826: OR=0.714, 95% CI: 0.608–0.838 $p<0.001$; rs9535828: OR=0.679, 95% CI: 0.579–0.796, $p<0.001$). The frequencies of rs10981694-TG/GG and rs10488764-GA/AA genotypes were significantly higher in lung cancer cases than that in controls, making them risk genotypes for the disease ($p < 0.001$); while the rs9535826-TG/GG and rs9535828-GA/AA genotypes were protective genotypes and associated with a reduced risk of the disease ($p<0.001$). Genetic model evaluation revealed that *SLC31A1*-rs10981694 and *FDX1*-rs10488764 were associated with a growing risk of lung cancer in dominant, recessive and log-additive models ($p<0.001$). Moreover, rs9535826 and rs9535828 in *ATP7B* were related to a declining risk of the disease in three genetic models ($p<0.001$). In addition, stratification analysis showed that *FDX1*-rs10488764 was risk variant for lung cancer in both smokers and nonsmokers, and was associated with risk of each pathological type of lung cancer ($p<0.008$).

Conclusion: The results shed new light on the correlation between cuproptosis-related genes and risk of lung cancer.

Keywords: lung cancer, single-nucleotide polymorphisms, SNPs, solute carrier family 31 member 1, *SLC31A1*, ferredoxin 1, *FDX1*, ATPase copper transporting beta, *ATP7B*

Introduction

Lung cancer is a malignant tumor with the highest morbidity and mortality in China.[1,2] The early stage of lung cancer generally has no specific clinical manifestations. Almost 70% of the patients were diagnosed at an advanced stage and even with distant metastasis, and lost the best treatment chance.[3] Therefore, early detection, diagnosis, and treatment is the key to reducing the mortality and improving prognosis of the disease. Sufficient research evidence has identified a number of risk factors for lung cancer, including smoking, second-hand smoke, occupational exposure to asbestos and silica, indoor and atmospheric air pollution, and so on.[4,5] At the same time, with the wide application of molecular biology technology in recent years, the effect of individual gene susceptibility on the risk of lung cancer has also been verified.[6,7] The genetic predisposition to lung cancer is mainly involved in the high-frequency low-penetrance mutation caused by single-nucleotide polymorphisms (SNPs) and low-frequency high-penetrance mutation caused by driver gene

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mutation.[8] Therefore, in-depth development and exploration of SNPs is helpful to screen genetic high-risk group and provide them genetic counseling, and therefore contributing to the early detection and diagnosis of the lung cancer.

Cuproptosis is a copper-dependent and mitochondrial respiration-related cell death, which is different from known death mechanisms such as apoptosis, pyroptosis and ferroptosis.[9] The copper level was found to be increased in lung cancer patients, which could promote tumor angiogenesis, progression and metastasis.[10,11] Therefore, investigation of cuproptosis-related genes in patients with lung cancer could be of great significance. A recent study has found that cuproptosis was realized by the combination of copper and lipoxylated components in the cycle of tricarboxylic acid, which led to the lipoxylated protein aggregation and subsequent iron–sulfur cluster protein loss, resulting in protein toxic stress and cell death.[12] The ferredoxin 1 (*FDX1*) encodes a small iron–sulfur protein that transfers electrons from NADPH through ferredoxin reductase to mitochondrial cytochrome P450, which is an upstream regulator for lipoxylation and essential for copper ionophore–induced cell death.[12] The solute carrier family 31 member 1 (SLC31A1) is a high-affinity copper transporter in the cell membrane, function as a homotrimer to affect the uptake of dietary copper.[13] In addition, ATPase copper transporting beta (ATP7B) generally acts as a copper-transporting ATPase which exports copper out of the cells.[14] Previous studies mainly focused on the role of these three genes in copper metabolism disorder (Wilson disease), and the platinum resistance in cancer patients treated with platinum drugs.[15,16] However, little research evaluated the association between SNPs in the three genes and risk of lung cancer.

Considering the essential role exerted by copper and cuproptosis in the onset and development of lung cancer, we selected six SNPs on *SLC31A1, FDX1* and *ATP7B* based on the previous studies, and genotyped these polymorphisms in our case–control cohort, and assessed their association with risk of lung cancer. rs2233914 in *SLC31A1* was related to better prognosis and longer survival time in lung cancer patients treated with platinum drugs.[17] rs10981694 in *SLC31A1* was correlated with cisplatin-related toxicity in lung cancer patients after cisplatin treatment.[18] Moreover, *FDX1*-rs10488764-AA genotype was found to be associated with an elevated risk of IgA nephropathy.[19] In addition, rs1061472, rs9535826 and rs9535828 in *ATP7B* were investigated in the gastrointestinal toxicity of lung cancer patients treated with platinum-based chemotherapy.[20,21] None of these studies directly evaluated the associations between these SNPs and risk of lung cancer, especially in different pathological types. We hope our genotyping results could provide new clues for the role of cuproptosis-related genes in the pathogenesis of lung cancer.

Materials and Methods

Subjects

A total of 650 lung cancer patients and 650 healthy controls were included in this study. All subjects were of Chinese Han ethnicity and were recruited at Tangdu Hospital. The patients were diagnosed with lung cancer by histopathological examination of biopsy specimens. The control group included randomly selected healthy individuals with no history of cancer. All participants provided written informed consent. This study was approved by the Ethics Committee of Tangdu Hospital and carried out in accordance with the World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects.

Genotyping

Six SNPs in the cuproptosis-related genes *SLC31A1, FDX1* and *ATP7B* were chosen for genotyping based on previous association studies. The minor allele frequencies (MAFs) of these SNPs are >5% in East Asian populations according to the NCBI database. DNA was extracted using a QIAamp DNA Blood Midi Kit (QIAGEN, Germany). Primers were designed using Sequenom MassARRAY Assay Design 3.0 software. SNP genotyping was performed on a Mass ARRAY iPLEX platform (Sequenom, San Diego, CA, USA).

Statistical Analysis

Statistical analysis was performed with SPSS package version 20.0 (SPSS, Chicago, IL, USA). The MAFs of each SNP were checked for divergence from the Hardy–Weinberg equilibrium (HWE). HaploReg v4.1 (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) was used to predict the potential functions of the SNPs. Allele and genotype frequencies in the cases and controls were evaluated using Chi-square tests. The association between SNPs and lung cancer risk was evaluated using SNPstats (https://www.snpstats.net/start.htm) and expressed by odds ratios (ORs) and

95% confidence intervals (CIs) with adjustments for sex, age and smoking status. All p values were Bonferroni corrected, and statistical significance was set at $p \leq 0.008$ (0.05/6).

Results

The demographic characteristics of the participants are listed in Table 1, including sex, age, smoking status and pathological types. No significant differences were observed in the distributions of sex, age and smoking status between the case and control groups ($p > 0.05$). The pathological types of cases mainly included adenocarcinoma, squamous cell carcinoma and small cell lung cancer, with a percentage of 46.2%, 31.2% and 18.8%, respectively. In addition, 3.8% of the patients were other types of lung cancer.

The basic information and predicted functions of candidate SNPs are described in Table 2. The predicted function according to the HaploReg database showed that rs2233914 and rs10981694 in *SLC31A1*, rs10488764 in *FDX1*, and

Table 1 The Demographic Characteristics of the Participants

Characteristics	Case (n=650)	Control (n=650)	χ^2/t	*p*
Sex (%)			0.030	0.862
Male	418 (64.3)	415 (63.8)		
Female	232 (35.7)	235 (36.2)		
Age			0.688	0.337
Mean ±SD	56.91±10.17	56.36±10.26		
Smoking (%)			0.030	0.862
Yes	415 (63.8)	412 (63.4)		
No	235 (36.2)	238 (36.6)		
Pathological types				
Adenocarcinoma	300 (46.2)			
Squamous cell carcinoma	203 (31.2)			
Small cell lung cancer	122 (18.8)			
Others	25 (3.8)			

Table 2 Basic Information and Predicted Functions of Candidate SNPs

SNP	Gene	Position	Allele	Region	Predicted Functions
rs2233914	*SLC31A1*	chr9:113221260	G>A	2kB Upstream Variant	Promoter histone marks, DNAse, Motifs changed, eQTLhits
rs10981694	*SLC31A1*	chr9:113224129	T>G	Intron Variant	DNAse, Motifs changed, Selected eQTLhits
rs10488764	*FDX1*	chr11:110460907	G>A	Intron Variant	Motifs changed, eQTLhits
rs1061472	*ATP7B*	chr13:51950352	T>C	Missense Variant	Lys832Arg
rs9535826	*ATP7B*	chr13:51991990	T>G	Intron Variant	Enhancer histone marks, Motifs changed, eQTLhits
rs9535828	*ATP7B*	chr13:51999286	G>A	Intron Variant	Promoter/Enhancer histone marks, DNAse, Motifs changed, eQTLhits

Abbreviations: SNP, single-nucleotide polymorphism; eQTL, expression quantitative trait locus.

rs9535826 and rs9535828 in *ATP7B* were involved in promoter/enhancer histone marks, DNAse, motifs changed and eQTLhits, making it a potential function on the regulation of the gene expression. Moreover, rs1061472 in *ATP7B* was a missense variant, and led to Lys832Arg.

The MAFs of candidate SNPs between cases and controls are presented in Table 3. All of the SNPs were consistent with HWE ($p > 0.05$). We compared the MAF of each SNP between the two groups and found that two SNPs were associated with an increased risk of lung cancer, and other two SNPs were protective factors for the disease. The minor alleles of *SLC31A1*-rs10981694 and *FDX1*-rs10488764 were associated with a 1.455-fold and 1.483-fold increased risk of lung cancer, respectively (rs10981694: 95% CI: 1.201–1.763, $p<0.001$; rs10488764: 95% CI: 1.244–1.768, $p<0.001$). In contrast, the minor alleles of rs9535826 and rs9535828 in *ATP7B* were related to a decreased risk of the disease (rs9535826: OR=0.714, 95% CI: 0.608–0.838 $p<0.001$; rs9535828: OR=0.679, 95% CI: 0.579–0.796, $p<0.001$).

The genotype frequency distributions between cases and controls are shown in Table 4. The frequencies of rs10981694-TG/GG genotypes were significantly higher in lung cancer cases than that in controls, making them risk genotypes for the disease ($p = 0.0005$). Similarly, the rs10488764-GA/AA genotypes were also related to an elevated risk of lung cancer ($p<0.0001$). By contrast, the frequencies of rs9535826-TG/GG and rs9535828-GA/AA genotypes were lower in cases than in controls, which made them become protective genotypes and associated with a reduced risk of the disease ($p_{rs9535826}$ =0.0002, $p_{rs9535828}$ <0.0001).

The effect of SNPs on the risk of lung cancer was further evaluated using three genetic models (Table 5). The results were consistent with allelic and genotypic results. The *SLC31A1*-rs10981694 and *FDX1*-rs10488764 were associated with a growing risk of lung cancer in dominant, recessive and log-additive models (rs10981694: p_{dominant} =0.0005, $p_{\text{recessive}}$ =0.0085, $p_{\text{log-additive}}$ =0.0001; rs10488764: p_{dominant} <0.0001, $p_{\text{recessive}}$ =0.006, $p_{\text{log-additive}}$ <0.0001). In addition, rs9535826 and rs9535828 in *ATP7B* were related to a declining risk of the disease in three genetic models (rs9535826: p_{dominant} =0.0002, $p_{\text{recessive}}$ =0.0043, $p_{\text{log-additive}}$ <0.0001; rs9535828: p_{dominant} <0.0001, $p_{\text{recessive}}$ =0.0002, $p_{\text{log-additive}}$ <0.0001).

Considering that smoking could be a potential risk factor and the different pathogenesis in various pathological types of lung cancer, stratification analysis according to smoking status and different pathological types were further performed (Tables 6 and 7). The *FDX1*-rs10488764 remained risk variant for lung cancer in both smokers and nonsmokers, and was associated with risk of each pathological type of lung cancer ($p<0.008$). In addition, the rs9535828 in *ATP7B* was still a protective factor for the disease whether smoking or not ($p<0.008$). However, *SLC31A1*-rs10981694 was only associated with squamous cell carcinoma and small cell lung cancer ($p<0.008$), and rs9535826 was not a protective variant for the risk of squamous cell carcinoma, which may be due to the limited sample size or the different pathogenesis.

Table 3 The MAF and HWE of Candidate SNPs Between Lung Cancer Cases and Healthy Controls

SNP	Gene	MAF-Case	MAF-Control	HWE *p*	OR (95% CI)	*p*
rs2233914	*SLC31A1*	0.35	0.33	0.86	1.075(0.914–1.264)	0.385
rs10981694	*SLC31A1*	0.24	0.17	0.22	1.455(1.201–1.763)	0.00012*
rs10488764	*FDX1*	0.30	0.23	0.57	1.483(1.244–1.768)	0.00001*
rs1061472	*ATP7B*	0.41	0.40	0.46	1.066(0.911–1.247)	0.424
rs9535826	*ATP7B*	0.32	0.40	0.19	0.714(0.608–0.838)	0.00004*
rs9535828	*ATP7B*	0.34	0.43	0.75	0.679(0.579–0.796)	0.00001*

Note: *Bonferroni multiple adjustment was applied, with $p \leq 0.008$.
Abbreviations: SNP, single-nucleotide polymorphism; MAF, minor allele frequency; HWE, Hardy–Weinberg equilibrium.

Table 4 Genotype Frequency Distributions Between Lung Cancer Cases and Healthy Controls

Model	Genotype	Control	Case	OR (95% CI)	*p*
rs2233914	GG	290 (44.6%)	269 (41.4%)	1	0.47
	GA	287 (44.1%)	308 (47.4%)	1.15 (0.92–1.46)	
	AA	73 (11.2%)	73 (11.2%)	1.08 (0.75–1.55)	
rs10981694	TT	438 (67.4%)	377 (58%)	1	0.0005*
	TG	197 (30.3%)	240 (36.9%)	1.44 (1.12–1.84)	
	GG	15 (2.3%)	33 (5.1%)	2.57 (1.37–4.82)	
rs10488764	GG	392 (60.3%)	318 (48.9%)	1	<0.0001*
	GA	222 (34.1%)	271 (41.7%)	1.52 (1.21–1.92)	
	AA	36 (5.5%)	61 (9.4%)	2.15 (1.39–3.34)	
rs1061472	TT	242 (37.2%)	226 (34.8%)	1	0.67
	TC	302 (46.5%)	314 (48.3%)	1.11 (0.87–1.42)	
	CC	106 (16.3%)	110 (16.9%)	1.10 (0.80–1.53)	
rs9535826	TT	224 (34.5%)	291 (44.8%)	1	0.0002*
	TG	329 (50.6%)	296 (45.5%)	0.69 (0.55–0.88)	
	GG	97 (14.9%)	63 (9.7%)	0.50 (0.35–0.72)	
rs9535828	GG	207 (31.9%)	285 (43.9%)	1	<0.0001*
	GA	324 (49.9%)	287 (44.1%)	0.49 (0.37–0.64)	
	AA	119 (18.3%)	78 (12%)	0.30 (0.20–0.45)	

Note: *Bonferroni multiple adjustment was applied, with $p \leq 0.008$.
Abbreviations: SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

Discussion

Copper and cuproptosis is closely related to the genesis, severity, and progression of cancer, making it a vulnerable point to target for cancer prevention and treatment.[22] In this study, we focused on cuproptosis-related gene polymorphisms in lung cancer patients and healthy controls, and identified two risk variants (*SLC31A1*-rs10981694 and *FDX1*-rs10488764) and two protective mutations (rs9535826 and rs9535828 in *ATP7B*) for lung cancer. The results broadened our knowledge on the effects of cuproptosis-related gene polymorphisms on the risk of lung cancer and provided new clues for the screening of high-risk population and early detection and diagnosis of the disease.

SLC31A1 encodes the copper transporter 1 (CTR1) that belongs to the copper transporter family, playing an essential role in regulating the copper homeostasis and affecting the cisplatin and carboplatin uptake in human cells.[23,24] Barresi et al reported that the mRNA level of *SLC31A1* was significantly increased in colorectal carcinoma samples, which was accompanied by a series of elevated expression of copper metabolism-related genes, such as *ATP7A*, *SCO1* and *COX11*.[25] Moreover, the high levels of *SLC31A1* were successively found in prostate cancer, hepatocellular carcinoma and pancreatic cancer, which drew researchers' attention on the role of *SLC31A1* in cancer development.[26–28] Yu et al found that inhibition of *SLC31A1* and blockage of copper absorption caused an elevated mitochondrial ROS level and reduced ATP level in pancreatic cancer cells, and led to an increased autophagy to resist the cell death.[28] In addition, Wu et al demonstrated that ZNF711 could recruit the JHDM2A to the promoter and *SLC31A1* and activate its expression, resulting in an enhancement of cisplatin uptake in epithelial ovarian cancer.[29] As for the polymorphisms in *SLC31A1*, Fujita et al identified that rs10981694 A>C was correlated with a poorer prognosis in esophageal cancer patients treated with neoadjuvant chemoradiotherapy.[16] Wang et al revealed that *SLC31A1* rs2233914 has an interaction

Table 5 Association Between SNPs and Risk of Lung Cancer in Genetic Models

SNP	Model	Genotype	Control	Case	OR (95% CI)	*p*
rs2233914	Dominant	GG	290 (44.6%)	269 (41.4%)	1	0.250
		GA-AA	360 (55.4%)	381 (58.6%)	1.14 (0.91–1.42)	
	Recessive	GG-GA	577 (88.8%)	577 (88.8%)	1	1.000
		AA	73 (11.2%)	73 (11.2%)	1.00 (0.71–1.41)	
	Log-additive	—	—	—	1.08 (0.91–1.27)	0.390
rs10981694	Dominant	TT	438 (67.4%)	377 (58%)	1	0.0005*
		TG-GG	212 (32.6%)	273 (42%)	1.52 (1.20–1.93)	
	Recessive	TT-TG	635 (97.7%)	617 (94.9%)	1	0.0085*
		GG	15 (2.3%)	33 (5.1%)	2.24 (1.20–4.16)	
	Log-additive	—	—	—	1.50 (1.22–1.84)	0.0001*
rs10488764	Dominant	GG	392 (60.3%)	318 (48.9%)	1	<0.0001*
		GA-AA	258 (39.7%)	332 (51.1%)	1.61 (1.29–2.01)	
	Recessive	GG-GA	614 (94.5%)	589 (90.6%)	1	0.006*
		AA	36 (5.5%)	61 (9.4%)	1.81 (1.18–2.78)	
	Log-additive	—	—	—	1.49 (1.25–1.78)	<0.0001*
rs1061472	Dominant	TT	242 (37.2%)	226 (34.8%)	1	0.370
		TC-CC	408 (62.8%)	424 (65.2%)	1.11 (0.88–1.39)	
	Recessive	TT-TC	544 (83.7%)	540 (83.1%)	1	0.790
		CC	106 (16.3%)	110 (16.9%)	1.04 (0.78–1.39)	
	Log-additive	—	—	—	1.06 (0.91–1.24)	0.450
rs9535826	Dominant	TT	224 (34.5%)	291 (44.8%)	1	0.0002*
		TG-GG	426 (65.5%)	359 (55.2%)	0.65 (0.52–0.81)	
	Recessive	TT-TG	553 (85.1%)	587 (90.3%)	1	0.0043*
		GG	97 (14.9%)	63 (9.7%)	0.61 (0.44–0.86)	
	Log-additive	—	—	—	0.70 (0.60–0.83)	<0.0001*
rs9535828	Dominant	GG	207 (31.9%)	285 (43.9%)	1	<0.0001*
		GA-AA	443 (68.2%)	365 (56.1%)	0.46 (0.35–0.60)	
	Recessive	GG-GA	531 (81.7%)	572 (88%)	1	0.0002*
		AA	119 (18.3%)	78 (12%)	0.53 (0.38–0.74)	
	Log-additive	—	—	—	0.54 (0.44–0.65)	<0.0001*

Note: *Bonferroni multiple adjustment was applied, with $p \leq 0.008$.
Abbreviations: SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

with *ABCG2* rs1871744, which are associated with poor response in lung cancer patients receiving platinum-based chemotherapy.[30] In this study, we genotyped rs10981694 and rs2233914 polymorphisms in our case–control cohort, and found that *SLC31A1*-rs10981694 is an independent risk variant for each pathological type of lung cancer, suggesting its

Table 6 Association Between rs10981694, rs10488764, rs9535826 and rs9535828 and Risk of Lung Cancer in Smokers and Nonsmokers

SNP	Model	Genotype	Smokers		Nonsmokers	
			OR (95% CI)	*p*	OR (95% CI)	*p*
rs10981694	Dominant	TT	1	0.057	1	0.0016*
		TG-GG	1.45 (1.02–2.08)		1.86 (1.26–2.73)	
	Recessive	TT-TG	1	0.040	1	0.048
		GG	1.99 (0.92–4.31)		2.72 (0.95–7.77)	
	Log-additive	—	1.42 (1.06–1.90)	0.018	1.79 (1.27–2.52)	0.0006*
rs10488764	Dominant	GG	1	0.010	1	0.0034*
		GA-AA	1.61 (1.21–2.14)		1.75 (1.20–2.55)	
	Recessive	GG-GA	1	0.240	1	0.0039*
		AA	1.39 (0.80–2.41)		2.68 (1.33–5.40)	
	Log-additive	—	1.44 (1.14–1.81)	0.002*	1.66 (1.24–2.21)	0.0005*
rs9535826	Dominant	TT	1	0.028	1	0.0008*
		TG-GG	0.73 (0.55–0.97)		0.52 (0.36–0.77)	
	Recessive	TT-TG	1	0.064	1	0.027
		GG	0.66 (0.43–1.03)		0.55 (0.32–0.94)	
	Log-additive	—	0.76 (0.62–0.94)	0.012	0.61 (0.47–0.81)	0.0004*
rs9535828	Dominant	GG	1	<0.0001*	1	0.100
		GA-AA	0.27 (0.18–0.40)		0.72 (0.49–1.06)	
	Recessive	GG-GA	1	0.004*	1	0.0087*
		AA	0.50 (0.32–0.81)		0.53 (0.32–0.86)	
	Log-additive	—	0.32 (0.24–0.45)	<0.0001	0.72 (0.55–0.93)	0.011

Note: *Bonferroni multiple adjustment was applied, with $p \leq 0.008$.
Abbreviations: SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

important role in the onset of the disease in addition to the cisplatin resistance. Considering the function of *SLC31A1* in copper transport and cuproptosis, we supposed that rs10981694 may alter the normal function of *SLC31A1* and the cuproptosis in patients with lung cancer. However, the detailed mechanisms need to be explored in the further studies.

FDX1 and FDX2 are two homologous ferredoxins in the human mitochondria. Previous studies on the function of these two ferredoxins has been controversial. Sheftel et al reported that FDX1 and FDX2 had distinct roles: FDX1 only participated in the biosynthesis of steroid hormones, whereas FDX2 contributed to the production of heme A and Fe−S cluster formation.[31] Subsequently, Shi et al found that knock-out of FDX1 decreased the enzyme activity of iron–sulfur cluster and affected iron homeostasis, and demonstrated that both FDX1 and FDX2 were closely involved in the formation of Fe−S cluster.[32] Cai et al further proved the important function of FDX1 in the biosynthesis process of Fe−S cluster using nuclear magnetic resonance spectroscopy.[33] More recently, Tsvetkov identified FDX1 could rescue the cell death induced by elesclomol using CRISPR-Cas9 screening, and further revealed that FDX1 specifically promoted the copper-dependent cell death.[34] Specifically, FDX1 could target the six important components in lipoic acid pathway, including LIPT1, LIAS, DLD, DLAT, PDHA1 and PHDB; and it is also a key mediator of protein lipoylation, making it an important promoting factor for cuproptosis.[12] However, little information is found about the correlation between FDX1 and lung cancer. Zhang

Table 7 Association Between rs10981694, rs10488764, rs9535826 and rs9535828 and Risk of Different Pathological Types of Lung Cancer

SNP	Model	Genotype	Adenocarcinoma		Squamous Cell Carcinoma		Small Cell Lung Cancer	
			OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
rs10981694	Dominant	TT	1	0.048	1	0.0001*	1	0.024
		TG-GG	1.35 (1.00–1.81)		2.06 (1.44–2.96)		1.67 (1.07–2.59)	
	Recessive	TT-TG	1	0.027	1	0.07	1	0.015
		GG	2.31 (1.11–4.83)		2.21 (0.96–5.09)		3.26 (1.34–7.93)	
	Log-additive	—	1.37 (1.07–1.77)	0.014	1.86 (1.38–2.53)	0.0001*	1.70 (1.18–2.46)	0.005*
rs10488764	Dominant	GG	1	0.0021*	1	0.0098	1	0.0006*
		GA-AA	1.55 (1.17–2.05)		1.53 (1.11–2.12)		2.00 (1.34–2.96)	
	Recessive	GG-GA	1	0.22	1	0.0006*	1	0.058
		AA	1.42 (0.82–2.45)		2.70 (1.56–4.68)		1.97 (1.01–3.85)	
	Log-additive	—	1.40 (1.12–1.75)	0.003*	1.56 (1.22–2.00)	0.0005*	1.72 (1.27–2.31)	0.0005*
rs9535826	Dominant	TT	1	0.0013*	1	0.18	1	0.0048*
		TG-GG	0.63 (0.47–0.83)		0.80 (0.57–1.11)		0.56 (0.38–0.84)	
	Recessive	TT-TG	1	0.006*	1	0.29	1	0.015
		GG	0.54 (0.34–0.85)		0.77 (0.47–1.26)		0.44 (0.22–0.91)	
	Log-additive	—	0.67 (0.54–0.83)	0.0002*	0.83 (0.65–1.06)	0.13	0.60 (0.44–0.82)	0.0011*
rs9535828	Dominant	GG	1	<0.0001*	1	0.0022*	1	<0.0001*
		GA-AA	0.46 (0.33–0.64)		0.52 (0.34–0.79)		0.36 (0.22–0.59)	
	Recessive	GG-GA	1	0.0041*	1	0.0035*	1	0.049
		AA	0.55 (0.36–0.84)		0.48 (0.28–0.80)		0.53 (0.27–1.03)	
	Log-additive	—	0.55 (0.43–0.71)	<0.0001*	0.52 (0.38–0.72)	<0.0001*	0.46 (0.31–0.67)	<0.0001*

Note: *Bonferroni multiple adjustment was applied, with $p \leq 0.008$.
Abbreviations: SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

et al reported that FDX1 was not directly related to cell growth or apoptosis, but it did promote the ATP production and take part in the metabolism of glucose, fatty acid and amino acid in lung adenocarcinoma.[35] In the present study, we determined that *FDX1*-rs10488764 was a risk polymorphism for lung cancer in both smokers and nonsmokers, and three different pathological types subgroups, which shed new light on the role of *FDX1* on the development of the disease.

ATP7B is an essential copper-transporting protein that regulates copper transportation from cytosol to Golgi apparatus or lysosomes to maintain copper homeostasis.[36] Generally, ATP7B transfers copper to the Golgi network, whereas the high copper level alters the localization of ATP7B to lysosomes, resulting in a release of copper by vesicle transport.[37] Yang et al reported that the expression of ATP7B was significantly correlated with tumor cell differentiation in lung cancer.[38] Moreover, Li et al demonstrated that ATP7B expression was closely linked to the overall survival and treatment response in lung cancer patients receiving platinum-based chemotherapy.[39] As for polymorphisms in *ATP7B*, most of studies focused on its association with chemotherapeutic drug response in patients with cancer. In the early stage, Fukushima-Uesaka et al detected a total of 61 genetic variations on *ATP7B* in Japanese cancer patients and provided reference allele frequencies for other similar studies on Asian populations.[40] Subsequently, Schmid et al identified that loss of heterozygosity of the *ATP7B* exhibited a better response in patients

with bladder cancer after platinum-based chemotherapy.[41] In addition, Li et al genotyped ATP7B rs1061472 and rs9535826 polymorphisms on *ATP7B* in 427 lung cancer patients and reported that individuals with rs9535826-GG genotype exhibited a lower gastrointestinal toxicity after platinum-based chemotherapy.[20] We genotyped rs1061472, rs9535826 and rs9535828 on *ATP7B* in our case–control cohort and found that rs9535826 and rs9535828 were independent protective factors the lung cancer. Considering the overload copper status in cancer, we supposed that rs9535826 and rs9535828 polymorphisms may be essential for maintain the normal function of *ATP7B* and copper homeostasis.

Recently, the latest association studies on lung cancer have provided us some novel research directions. Ji et al have reported that rs1948915 in lncRNA CCAT1 was correlated with risk of lung adenocarcinoma.[42] Liu et al have found that EGFL7/miR-126 polymorphism rs2297538 was associated with the risk of non-small cell lung cancer.[43] In addition, Yu et al have identified a novel regQTL-SNP, rs3768617, may have effects on lung cancer risk by influencing the expression of miRNA-548b-3p and LAMC1.[44] These studies remind us that we could also explore the association between SNPs in cuproptosis-related lncRNA, miRNA and lung cancer risk, and might identify some novel regQTL-SNP related to lung cancer risk in further studies. There are some intrinsic in our study. Firstly, the subjects were enrolled in a very long time period, we did not detect the copper level of the subjects from the very beginning; therefore, the interaction between polymorphisms in the three genes and the copper level could not be evaluated. Secondly, there are many other potential risk factors for lung cancer, such as alcohol consumption, occupational exposure and air pollution. We did not evaluate the interaction between these factors and candidate SNPs due to the limited information. Thirdly, the present association study could only provide clues for the association between polymorphisms in *SLC31A1, FDX1* and *ATP7B* and risk of lung cancer, but not fully reveal the underlying mechanism. The detailed molecular mechanism needs to be confirmed in tissue samples, cell experiments and animal models.

In conclusion, we found that *SLC31A1*-rs10981694 and *FDX1*-rs10488764 were associated with an elevated risk of lung cancer, while rs9535826 and rs9535828 in *ATP7B* were related to a declining risk of the disease. The results shed new light on the correlation between cuproptosis-related genes and risk of lung cancer, and provided novel reference information for the early detection and diagnosis of the disease.

Disclosure

The authors report no conflicts of interest in this work.

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