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Genetic variants in LKB1/AMPK/mTOR pathway are associated with clinical outcomes of chemotherapy in non-small cell lung cancer

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Abstract

This study was conducted to investigate the relationship between genetic variants in LKB1/AMPK/mTOR pathway and treatment outcomes of patients with non-small cell lung cancer (NSCLC) treated with chemotherapy. A total of 379 patients with NSCLC who underwent first-line paclitaxel-cisplatin chemotherapy was enrolled. The associations between 19 single nucleotide variants (SNVs) in the LKB1/AMPK/mTOR pathway and the chemotherapy response and overall survival (OS) were analyzed. Among the SNVs analyzed, *AKT1* rs2494750G>C and *TSC1* rs2809244C>A were associated with clinical outcomes after chemotherapy in multivariate analyses. The *AKT1* rs2494750G>C was significantly associated with a better response to chemotherapy (adjusted odds ratio [aOR]: 1.92, 95% confidence interval [CI]: 1.02–3.62, $p = 0.04$). The *TSC1* rs2809244C>A were significantly associated with better OS (adjusted hazard ratio [aHR]: 0.79, 95% CI: 0.62–0.99, $p = 0.04$). When stratified by tumor histology, *AKT1* rs2494750G>C exhibited a significant association with the chemotherapy response only in adenocarcinoma and *TSC1* rs2809244C>A was also significantly associated with OS only in adenocarcinoma. This result suggests that the *AKT1* rs2494750G>C and *TSC1* rs2809244 C>A may be useful for predicting the clinical outcome of first-line paclitaxel-cisplatin chemotherapy in NSCLC.

KEYWORDS
chemotherapy response, LKB1/AMPK/mTOR pathway, lung cancer, survival, variant

Sun Ha Choi and Sook Kyung Do are contributed equally to this work.

INTRODUCTION

Lung cancer is the leading cause of cancer-related death worldwide, with an average 5-year survival rate of 22%.[1] Non-small cell lung cancer (NSCLC) comprises 85% of all lung cancer, and two-thirds of which presents with locally advanced or metastatic disease at the time of diagnosis.[2] Over the last decade, targeted molecular therapies and immune checkpoint inhibitors have led to significant advances in the treatment of NSCLC.[3] However, the benefits of targeted agents have been confined to a subset of patients with NSCLC who have a suitable drug target, and only a proportion of patients obtain response to immune checkpoint inhibitors because of the diverse mechanisms of resistance.[3] Therefore, platinum-based chemotherapy, in combination with immune checkpoint inhibitors or alone, continues to play an important role in the treatment of advanced NSCLC. Because treatment outcome after chemotherapy varies widely among patients with similar clinical characteristics, there have been extensive investigations to identify molecular biomarkers that can better predict a patient's clinical outcome.

Liver kinase B1 (LKB1, also known as serine/threonine kinase 11, [STK11]) acts as a master upstream activator of adenosine monophosphate-activated protein kinase (AMPK), playing a crucial role in cell growth, polarity, and energy metabolism.[4,5] The LKB1/AMPK functions as a metabolic checkpoint in the cell, regulating cell growth and proliferation according to the availability of nutritional supplies, with a substantial part of this regulatory role being played through mammalian target of rapamycin (mTOR).[6,7] In addition, the LKB1/AMPK pathway plays a pivotal role in redox homeostasis by maintaining intracellular NADPH level.[8,9] Studies have suggested that LKB1 loss play an important role not only in tumorigenesis, but also in cancer invasion and metastasis.[10–13] Although LKB1 has been considered as a tumor suppressor, studies have revealed its potential oncogenic roles, suggesting its function as a double-edged sword. The metabolic checkpoint function of LKB1/AMPK may confer survival advantage for cancer cells under unfavorable conditions such as tumor microenvironment.[5,14,15] The loss of LKB1/AMPK signaling confers sensitivity to chemotherapy-induced oxidative stress on cancer cells and has been associated with an improved outcome in advanced NSCLC patients treated with chemotherapy.[16–18] LKB1 is involved in the DNA damage response and *LKB1* knockdown cells showed a reduced efficiency in the homologous repair machinery, sensitizing cells to DNA damaging treatments such as platinum compounds.[19,20] In addition, activation of autophagy by LKB1/AMPK could rescue cancer cells upon chemotherapy by degrading damaged cellular components, representing a mechanism of resistance.[16,21] Taken together, alterations in LKB1/AMPK and downstream pathway may modify the response of cancer cells to chemotherapy and clinical outcomes.

In this study, we investigated whether genetic variants in LKB1/AMPK and one of the major downstream signaling pathways regulated by LKB1/AMPK, the mTOR signaling, may have an impact on the clinical outcomes of chemotherapy in patients with NSCLC.

METHODS

Study populations

The study population was described in our previous studies.[22,23] Briefly, 379 patients with stage III or IV NSCLC, who underwent at least two cycles of paclitaxel-cisplatin chemotherapy as the first-line treatment at Kyungpook National University Hospital (KNUH) in Daegu, Korea between August 2005 and December 2008, were enrolled. Patients who underwent radiotherapy concurrently with chemotherapy as a first treatment modality were excluded to avoid the confounding effect of radiation on the response to chemotherapy. The chemotherapy regimen included 175 mg/m^2 paclitaxel administered intravenously over 3 h and 60 mg/m^2 cisplatin infused over 60 min on day 1 and every 3 weeks. Treatment was discontinued in cases of disease progression, major toxicities, or according to the decision of the patient or physician. The tumor response was assessed by computed tomography scanning every two cycles. Responses were evaluated using Response Evaluation Criteria in Solid Tumors. The best overall response for each patient was reported, and all responses were reviewed by an independent radiologist. Patients with a complete response (CR) or partial response (PR) were defined as responders, while patients with stable disease (SD) or progressive disease (PD) were defined as nonresponders. To assess survival outcomes, we recorded the overall survival (OS), defined as the time between the first date of chemotherapy and date of death or last follow-up. Genomic DNA samples from the patients were provided by the National Biobank of Korea, KNUH, which is supported by the Ministry of Health, Welfare, and Family Affairs. Written informed consent was obtained from all patients. This study was approved by the institutional review board of the KNUH and carried out in accordance with institutional review board-approved guidelines.

Selection of single-nucleotide variants (SNVs) and genotyping

To identify potentially functional variants in LKB1 pathway genes, we first searched a public SNP database (http://www.ncbi.nlm.nih.gov/SNP) for all SNPs in major LKB1/AMPK/mTOR pathway genes, and found a total of 720 variants in *AKT1*, *PIK3CA*, *PTEN*, *STK11*, *PRKAA1*, *PRKAA2*, *TSC1*, *TSC2*, and *mTOR* genes. Next, using the FuncPred utility for functional SNP prediction and TagSNP utility for linkage disequilibrium tag SNP selection in the SNPinfo web server (https://snpinfo.niehs.nih.gov), 23 potentially functional SNPs in seven genes with a minor allele frequency ≥0.05 in

TABLE 1 Univariate analysis for the response to chemotherapy and overall survival by clinical variables

Variables	No. of cases	Response to chemotherapy				Overall survival				
		Responders (CR + PR)	Nonresponders (SD + PD)	OR (95% CI)	*p*-value	MST (months)	95% CI	Log-rank *p*-value	HR (95% CI)	*p*-value
Overall	379	180 (47.5)[a]	199 (52.5)			13.2	12.5–14.7			
Age (years)										
<65	179	93 (52.0)	86 (48.0)	1.00		15.7	13.7–17.7		1.00	
≥65	200	87 (43.5)	113 (56.5)	0.71 (0.48–1.07)	0.10	11.9	10.8–13.2	0.003	1.38 (1.11–1.70)	0.003
Sex										
Male	309	153 (49.5)	156 (50.5)	1.00		12.8	11.9–14.3		1.00	
Female	70	27 (38.6)	43 (61.4)	0.64 (0.38–1.09)	0.10	16.8	12.8–22.6	0.02	0.73 (0.56–0.96)	0.02
Smoking status										
Never	63	27 (42.9)	36 (57.1)	1.00		19.6	13.7–30.4		1.00	
Ever	316	153 (48.4)	163 (51.6)	1.25 (0.73–2.16)	0.42	12.8	11.7–14.2	0.001	1.59 (1.20–2.12)	0.002
Histological type										
Squamous cell carcinoma	184	98 (53.3)	86 (46.7)	1.00		13.2	11.7–14.4		1.00	
Adenocarcinoma	172	71 (41.3)	101 (58.7)	0.62 (0.41–0.94)	0.02	15.1	12.1–17.5		0.74 (0.59–0.92)	0.01
NSCLC-NOS	23	11 (47.8)	12 (52.2)	0.80 (0.34–1.92)	0.62	11.4	7.4–12.9	0.01	1.25 (0.80–1.99)	0.34
Clinical stage										
III	159	82 (51.6)	77 (48.4)	1.00		14.7	12.8–17.4		1.00	
IV	220	98 (44.6)	122 (55.5)	0.75 (0.50–1.14)	0.18	12.7	10.8–14.2	0.12	1.18 (0.96–1.47)	0.12
PS ECOG										
0–1	310	149 (48.1)	161 (51.9)	1.00		14.1	12.6–15.7		1.00	
2	69	31 (44.9)	38 (55.1)	0.88 (0.52–1.49)	0.64	12.6	9.7–13.2	0.42	1.12 (0.85–1.48)	0.42
Weight loss										
No	233	116 (49.8)	117 (50.2)	1.00		14.4	12.5–16.6		1.00	
Yes	146	64 (43.8)	82 (56.2)	0.79 (0.52–1.19)	0.26	12.9	11.6–14.0	0.001	1.47 (1.18–1.83)	0.001
Second-line chemotherapy										
No	132					11.0	8.1–12.8		1.00	
Yes	247					15.1	13.2–16.6	0.02	0.76 (0.61–0.95)	0.02
Sequential radiotherapy										
No	340					12.9	11.6–14.3		1.00	
Yes	39					18.5	14.0–23.9	0.19	0.80 (0.57–1.12)	0.19

Abbreviations: CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; MST, median survival time; OR, odds ratio; PS, performance status.

[a]Row percentage.

the HapMap JPT data were collected after excluding those in linkage disequilibrium ($r^2 \geq 0.8$). Genomic DNA was extracted from peripheral blood lymphocytes using a blood QuickGene DNA whole blood kit S (Fujifilm). Genotyping was performed using the MassARRAY iPLEX assay (Sequenom Inc.).

Statistical analysis

We tested for Hardy–Weinberg equilibrium using a goodness-of-fit χ^2 test with 1 degree of freedom. The linkage disequilibrium among variants was measured by using HaploView (http://broad.mit.edu/mpg/haploview). The genotypes for each SNV were analyzed as a three-group categorical variable and analyzed under dominant, recessive, and additive genetic models. The association between clinical variables or genotypes and chemotherapy response was tested by determining the odds ratio (OR) and 95% confidence intervals (CIs) using unconditional logistic regression analysis. The Kaplan–Meier method was used to calculate survival estimates. The difference in OS, according to different clinical variables or genotypes, was compared using log-rank tests. Cox's proportional hazard regression model was used for multivariate survival analyses. The hazard ratio (HR) and 95% CI were also estimated. A cutoff *p*-value of 0.05 was adopted for all statistical analyses. Statistical data were obtained using the Statistical Analysis System for Windows, version 9.4 (SAS Institute).

TABLE 2 Summary of the association between analyzed 19 SNVs in LKB1/AMPK/mTOR pathway and the response to chemotherapy and overall survival

ID No.[a]	Gene	Location	Alleles	CR (%)	MAF	HWP	*p*-value for response to chemotherapy[b]			*p*-value for overall survival[c]		
							Dominant	Recessive	Codominant	Dominant	Recessive	Codominant
rs2494750	*AKT1*	nearGene-5	GC	96.0	0.371	0.642	0.21	0.04	0.06	0.22	0.75	0.30
rs2809244	*TSC1*	UTR-3	CA	96.0	0.404	0.109	0.32	0.07	0.10	0.04	0.97	0.17
rs1034528	*MTOR*	intron	GC	95.8	0.187	0.070	0.25	0.51	0.24	0.53	0.61	0.78
rs1050700	*TSC1*	UTR-3	AG	96.8	0.289	0.681	0.16	0.24	0.11	0.30	0.88	0.46
rs1057079	*MTOR*	cds-synon	AG	96.3	0.173	0.060	0.77	0.22	0.83	0.49	0.17	0.95
rs17036508	*MTOR*	intron	TC	94.7	0.116	0.535	0.92	0.85	0.97	0.77	0.60	0.90
rs3765904	*MTOR*	intron	TC	98.4	0.009	0.855	0.50		0.50	0.78		0.78
rs701848	*PTEN*	UTR-3	TC	96.0	0.449	0.926	0.74	0.09	0.45	0.25	0.64	0.63
rs857148	*PRKAA2*	UTR-3	GT	96.0	0.423	0.209	0.57	0.43	0.42	0.89	0.53	0.66
rs11121691	*MTOR*	cds-synon	CT	97.9	0.066	0.748	0.58	0.79	0.64	0.40	0.90	0.41
rs2074969	*TSC2*	intron	GA	97.9	0.202	0.487	0.11	0.52	0.11	0.94	0.93	0.97
rs3806317	*PRKAA2*	intron	TC	97.9	0.121	0.216	0.76	0.88	0.75	0.47	0.82	0.49
rs4962225	*TSC1*	nearGene-5	AC	97.9	0.116	0.315	0.18	0.98	0.10	0.99	0.13	0.83
rs7711806	*PRKAA1*	nearGene-5	TC	96.6	0.247	0.699	0.42	0.34	0.77	0.27	0.40	0.22
rs1342382	*PRKAA2*	UTR-3	AT	96.3	0.236	0.169	0.57	0.19	0.33	0.68	0.24	0.92
rs11581010	*PRKAA2*	intron	AG	95.0	0.107	0.112	0.90	0.74	0.83	0.57	0.65	0.74
rs4912411	*PRKAA2*	UTR-3	CA	97.9	0.403	0.870	0.58	0.83	0.79	0.42	0.82	0.50
rs9803799	*PRKAA2*	UTR-3	TG	95.0	0.158	0.685	0.13	0.10	0.40	0.39	0.97	0.45
rs1130214	*AKT1*	UTR-5	GT	94.5	0.119	0.630	0.53	0.34	0.41	0.50	0.99	0.55

Abbreviations: 3′UTR; three prime untranslated region; CR; call rate; MAF; minor allele frequency; HWP; Hardy–Weinberg equilibrium.

[a]Information about variants and ID were obtained from the NCBI database (http://ncbi.nih.gov).

[b]*p*-values were calculated using multivariate regression analysis, adjusted for age, sex, smoking status, tumor histology, stage, ECOG performance status and weight loss.

[c]*p*-values were calculated using multivariate Cox proportional hazard models, adjusted for age, sex, smoking status, tumor histology, stage, ECOG performance status, weight loss, second-line chemotherapy, and sequential radiotherapy.

TABLE 3 *AKT1* rs2494750 and *TSC1* rs2809244 and their associations with the response to chemotherapy and overall survival

Variant/ genotype	No. of cases (%)[a]	Response to chemotherapy				Overall survival		
		Responders (%)[b]	Non-responders (%)[b]	OR (95% CI)[c]	*p*-value[c]	L-R *p*-value	HR (95% CI)[d]	*p*-value[d]
AKT1 rs2494750								
GG	142 (39.0)	62 (43.7)	80 (56.3)	1.00		0.79	1.00	
GC	174 (47.8)	81 (46.6)	93 (53.5)	1.16 (0.73–1.84)	0.53		1.42 (0.81–2.49)	0.23
CC	48 (13.2)	29 (60.4)	19 (39.6)	2.08 (1.05–4.13)	0.04		1.14 (0.81–1.59)	0.45
Dominant				1.32 (0.85–2.04)	0.21	0.53	1.15 (0.92–1.44)	0.22
Recessive				1.92 (1.02–3.62)	0.04	0.96	1.05 (0.77–1.44)	0.75
Codominant				1.36 (0.99–1.86)	0.06		1.09 (0.93–1.27)	0.30
TSC1 rs2809244								
CC	122 (33.5)	53 (43.4)	69 (56.6)	1.00		0.20	1.00	
CA	190 (52.5)	87 (45.8)	103 (54.2)	1.13 (0.71–1.80)	0.62		0.77 (0.60–0.98)	0.03
AA	52 (14.3)	30 (57.7)	22 (42.3)	1.89 (0.96–3.70)	0.06		0.86 (0.61–1.21)	0.39
Dominant				1.25 (0.80–1.96)	0.32	0.09	0.79 (0.62–0.99)	0.04
Recessive				1.75 (0.96–3.22)	0.07	0.90	1.01 (0.74–1.38)	0.97
Codominant				1.31 (0.95–1.81)	0.10		0.89 (0.75–1.05)	0.17

Abbreviation: CI, confidence interval; HR, hazard ratio; L-R *p*, log-rank *p*; MST, median survival time (months); OR, odds ratio.
[a]Column percentage.
[b]Row percentage.
[c]ORs, 95% CI, and their corresponding *p*-values were calculated using multivariate regression analysis, adjusted for age, sex, smoking status, tumor histology, stage, ECOG performance status, and weight loss.
[d]HRs, 95% CIs and their corresponding *p*-values were calculated using multivariate Cox proportional hazard models, adjusted for age, sex, smoking status, tumor histology, stage, ECOG performance status, weight loss, second-line chemotherapy, and sequential radiotherapy.

RESULTS

Patient characteristics and clinical outcomes

The associations between clinicopathological features, chemotherapy response and OS are demonstrated in Table 1. The overall response rate was 47.5%. We observed deaths in 347 of the 379 patients (91.6%) and the median survival time (MST) was 13.2 months (95% CI: 12.5–14.7 months). Only tumor histology was significantly associated with response to chemotherapy. The OS was significantly associated with age, sex, smoking status, tumor histology, weight loss and second-line chemotherapy (Table 1).

Genotypes of *AKT1* rs2494750G>C and *TSC* rs2809244C>A and chemotherapy outcomes

Among the 23 SNVs genotyped, 19 were analyzed in this study after excluding four showing deviation from Hardy–Weinberg equilibrium ($p < 0.05$). The variant ID, gene information, miRNA, and minor allele frequencies are shown in Table 2. Among the 19 SNVs analyzed, *AKT1* rs2494750G>C and *TSC1* rs2809244C>A were associated with clinical outcomes after chemotherapy. The *AKT1* rs2494750G>C was significantly associated with better chemotherapy response under recessive model for the variant C allele (adjusted odds ratio [aOR]: 1.92, 95% confidence interval [CI]: 1.02–3.62, $p = 0.04$), but not significantly associated with OS (Table 3). The *TSC1* rs2809244C>A was significantly associated with better OS under dominant model for the variant A allele (adjusted hazard ratio [aHR]: 0.79, 95% CI: 0.62–0.99, $p = 0.04$), but there was no significant association with chemotherapy response (Table 3 and Figure 1a,b).

Effect of genetic variants on treatment outcomes according to tumor histology

Next, we analyzed the effect of the two variants on survival outcomes according to tumor histology (squamous cell carcinoma [SCC] vs. adenocarcinoma [AC]). When stratified by tumor histology, the *AKT1* rs2494750G>C and chemotherapy response was significantly associated only in AC (aOR: 2.20, 95% CI: 1.11–4.36, $p = 0.02$, under dominant model), but not in SCC (Table 4). The *TSC1* rs2809244C>A was also significantly associated with a better OS only in AC (aHR: 0.60, 95% CI: 0.42–0.86, $p = 0.01$, under dominant model) but not in SCC (Table 4 and Figure 1c–f). Although the association between *TSC1* rs2809244C>A and chemotherapy response was not significant in the overall patient population, subgroup analysis showed that *TSC1* rs2809244C>A was significantly associated with better

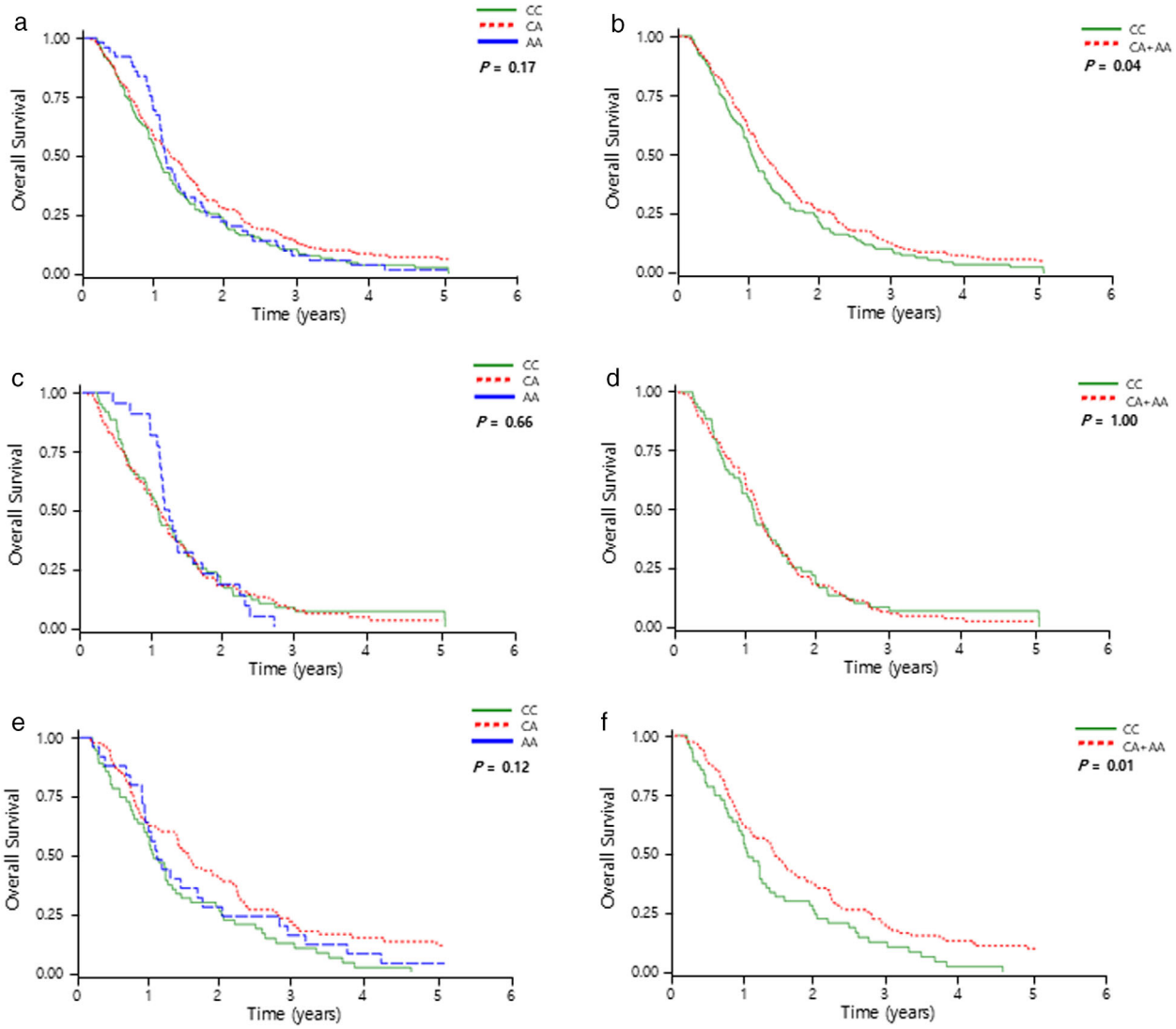

FIGURE 1 Overall survival curves according to *TSC1* rs2809244C>A in (a and b) all patients, (c and d) squamous cell carcinoma, and (e and f) adenocarcinoma. *p*-values by multivariate Cox proportional hazard models

chemotherapy response both in AC and SCC under different genetic models (aOR: 2.78, 95% CI: 1.35–5.72, $p = 0.01$, under dominant model; and aOR: 2.79, 95% CI: 1.08–7.23, $p = 0.03$, under recessive model, respectively) (Table 4).

DISCUSSION

In the present study, we investigated the association between genetic variants in the LKB1/AMPK/mTOR pathway and clinical outcomes of first-line paclitaxel-cisplatin chemotherapy in advanced NSCLC. Among the 19 variants evaluated, the *AKT1* rs2494750G>C was significantly associated with chemotherapy response and the *TSC1* rs2809244C>A was significantly associated with OS. Stratified analyses suggested that the genetic variants in this pathway might have different effects according to tumor histology. These findings suggest that the genetic variants in the LKB1/AMPK/mTOR pathway may be useful for predicting the clinical outcomes of NSCLC patients undergoing chemotherapy, helping to identify subgroups of patients who will benefit from chemotherapy.

Mammalian target of rapamycin (mTOR) signaling plays a central role in regulating protein synthesis, cell growth, proliferation, and survival.[24,25] The mTOR axis is one of the major downstream pathways of and is negatively regulated by the LKB1/AMPK signaling.[25] Phosphorylation of tuberous sclerosis complex 2 (TSC2) by AMPK after ATP depletion results in activation of the TSC1:TSC2 complex, which negatively regulates the activity of mTOR complex 1 (mTORC1).[26,27] The mTOR axis is activated by the phosphatidylinositol 3-kinase (PI3K)/AKT signaling.[24,25] AKT (also known as protein kinase B) plays a pivotal role in the PI3K-related signaling pathway, regulating cell survival, proliferation, and growth in response to many different growth factors.[28,29] Therefore, genetic

TABLE 4 The association between *AKT1* rs2494750 and *TSC1* rs2809244 and clinical outcomes in squamous cell carcinoma and adenocarcinoma

Variant/ histological subtype	Genotype	No. of cases (%)[a]	Response to chemotherapy					Overall survival	
			Responders (%)[b]	Nonresponders (%)[b]	OR (95% CI)[c]	*p*-value[c]	L-R *p*-value	HR (95% CI)[d]	*p*-value[d]
Akt1 rs2494750									
Squamous cell carcinoma	GG	66 (37.3)	36 (54.6)	30 (45.5)	1.00		0.74	1.00	
	GC	89 (50.3)	44 (49.4)	45 (50.6)	0.81 (0.42–1.55)	0.52		1.20 (0.85–1.68)	0.30
	CC	22 (12.4)	13 (59.1)	9 (40.9)	1.23 (0.45–3.39)	0.69		1.12 (0.68–1.85)	0.67
	Dominant				0.87 (0.47–1.63)	0.67	0.44	1.18 (0.85–1.63)	0.32
	Recessive				1.39 (0.54–3.56)	0.49	0.77	1.01 (0.64–1.60)	0.97
	Codominant				1.01 (0.63–1.59)	0.98		1.09 (0.87–1.37)	0.46
Adenocarcinoma	GG	64 (38.8)	20 (31.3)	44 (68.8)	1.00		0.76	1.00	
	GC	79 (47.9)	35 (44.3)	44 (55.7)	1.95 (0.95–4.02)	0.07		1.15 (0.79–1.66)	0.46
	CC	22 (13.3)	13 (59.1)	9 (40.9)	3.32 (1.19–9.28)	0.02		1.13 (0.67–1.93)	0.65
	Dominant				2.20 (1.11–4.36)	0.02	0.69	1.15 (0.81–1.63)	0.45
	Recessive				2.25 (0.89–5.67)	0.09	0.65	1.04 (0.64–1.70)	0.86
	Codominant				1.85 (1.14–3.02)	0.01		1.08 (0.85–1.39)	0.53
TSC1 rs2809244	CC	60 (34.1)	36 (60.0)	24 (40.0)	1.00		0.93	1.00	
Squamous cell carcinoma	CA	91 (54.7)	38 (41.8)	53 (58.2)	0.50 (0.25–0.99)	0.05		1.06 (0.74–1.53)	0.75
	AA	25 (14.2)	18 (72.0)	7 (28.0)	1.81 (0.64–5.15)	0.26		0.85 (0.51–1.41)	0.52
	Dominant				0.65 (0.34–1.24)	0.19	0.84	1.00 (0.71–1.41)	1.00
	Recessive				2.79 (1.08–7.23)	0.03	0.80	0.82 (0.52–1.31)	0.41
	Codominant				1.05 (0.66–1.67)	0.83		0.95 (0.75–1.20)	0.66
	CC	55 (32.9)	14 (25.5)	41 (74.6)	1.00		0.04	1.00	
Adenocarcinoma	CA	87 (52.1)	42 (48.3)	45 (41.7)	2.79 (1.32–5.90)	0.01		0.54 (0.37–0.79)	0.002
	AA	25 (15.0)	12 (48.0)	13 (52.0)	2.75 (1.01–7.47)	0.05		0.81 (0.50–1.33)	0.41
	Dominant				2.78 (1.35–5.72)	0.01	0.02	0.60 (0.42–0.86)	0.01
	Recessive				1.42 (0.60–3.36)	0.42	0.71	1.17 (0.74–1.83)	0.51
	Codominant				1.80 (1.11–2.91)	0.02		0.81 (0.62–1.05)	0.12

Abbreviations: CI, confidence interval; HR, hazard ratio; L-R *p*, log-rank *p*; OR, odds ratio.

[a]Column percentage.

[b]Row percentage.

[c]ORs, 95% CI, and their corresponding *p*-values were calculated by multivariate regression analysis, adjusted for age, sex, smoking status, stage, ECOG performance status, weight loss.

[d]HRs, 95% CI, and their corresponding *p*-values were calculated by multivariate Cox proportional hazard models, adjusted for age, sex, smoking status, stage, ECOG performance status, weight loss, second-line chemotherapy, and sequential radiotherapy.

alterations that cause activation of PI3K/AKT pathway or inactivation of LKB1/AMPK signaling may activate mTOR axis, resulting in inappropriate stimulation of protein translation and cell growth.

In the present study, among the evaluated genetic variants in the LKB1/AMPK/mTOR pathway, the *AKT1* rs2494750G>C and *TSC1* rs2809244C>A were significantly associated with clinical outcomes after paclitaxel-cisplatin chemotherapy in NSCLC. AKT signaling cascade is aberrantly activated in many human cancers, including lung cancer.[30,31] AKT activation is one of the contributing factors responsible for the development of cancer cells with increased resistance to a broad spectrum of chemotherapeutics and radiotherapy.[31–33] In addition, activated mTOR signaling acts as a mechanism of resistance to chemotherapy, and its inhibition has been shown to sensitize cancer cells to chemotherapy and reverse chemoresistance in several types of cancer.[34–37] Based on the role of TSC1:TSC2 complex in the negative regulation of mTORC1, it can be assumed that genetic alteration of *TSC1* may contribute to chemoresistance. Given that the *AKT1* rs2494750 and *TSC1* rs2809244 are located in 5′ near gene and 3′ untranslated region, respectively, these variants may affect the expression level of *AKT1* and *TSC1* by altered promoter activity or miRNA binding. Interestingly, stratified analysis suggested that variants in this pathway genes may have different predictive roles in different histological subtypes of NSCLC. Genetic alterations and pathogenesis in SCC and AC are markedly different, leading to the development of histology-specific therapeutics and different clinical outcomes.[38] Therefore, the predictors of clinical outcomes after a particular treatment may differ between SCC and AC.

However, there are several limitations in this study. First, the study cohort was relatively small and the *p*-values marginally reached statistical significance. Second, because all the subjects in the current study came from one country, the results may not be generalizable for other ethnic groups. Third, although stratified analyses suggested that the genetic variants in LKB1/AMPK/mTOR pathway might have different effects according to tumor histology, reduced sample sizes by dividing into two groups may have led to type II errors. Therefore, a well-designed and properly powered study including diverse populations of different ancestries is warranted to validate our findings. Lastly, this study did not include experiments to confirm the biological consequence of the variants. Further studies are necessary to understand the mechanism of association between these genetic variants and clinical outcomes.

In conclusion, this study showed that two genetic variants in LKB1/AMPK/mTOR pathway, the *AKT1* rs2494750G>C and *TSC1* rs2809244C>A, were associated with the clinical outcomes after paclitaxel-cisplatin chemotherapy in patients with advanced stage NSCLC. However, the results of this study need to be further tested in future studies for clinical validity and application.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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