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Prognostic Significance of STK11/LKB1 Expression and Its Role in the Tumor Microenvironment of Colorectal Adenocarcinoma

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Abstract

Background/Aim: The loss or mutation of serine–threonine kinase 11/liver kinase B1 (STK11/LKB1) is known to negatively impact prognosis and immunotherapy outcomes in non-small cell lung cancer (NSCLC), and germline mutations in this gene cause gastrointestinal adenocarcinomas in patients with Peutz–Jeghers syndrome (PJS). Although STK11/LKB1 mutations are rare in colorectal cancer, the down-regulation of STK11/LKB1 has been implicated in its tumorigenesis. However, the relationship between STK11/LKB1 expression and the immunosuppressive tumor microenvironment in colorectal cancer remains unclear.

Materials and Methods: In this study, we collected tissues from patients with colorectal adenocarcinoma (COAD) and constructed tissue microarrays (TMAs). STK11/LKB1 expression was assessed by immunohistochemistry and quantified by calculating H-scores. We also examined subsets of intratumoral tumor-infiltrating lymphocytes (TILs), including CD45+, CD8+, PD-1+, and CD45RO+ TILs, in these TMAs.

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Results: STK11/LKB1 expression was significantly correlated with nodal metastasis. Kaplan–Meier survival analysis demonstrated that low STK11 expression was associated with significantly poorer overall survival (OS), particularly in COAD patients with KRAS mutations. However, STK11/LKB1 expression was not correlated with the presence of intratumoral CD45+, CD8+, PD-1+, or CD45RO+ TILs. Finally, multivariate Cox proportional regression analysis identified STK11/LKB1 expression as an independent prognostic factor in COAD patients.
Conclusion: While STK11/LKB1 expression is associated with tumor progression and survival outcomes, there is no evidence that STK11/LKB1 expression influences the infiltration of lymphocytes into the tumor microenvironment.

Keywords: STK11/LKB1 expression, tumor microenvironment, prognosis, tumor-infiltrating lymphocytes, colorectal adenocarcinoma.

Introduction

Colorectal cancer is one of the most common malignant diseases worldwide. The development of colorectal cancer is driven by various gene mutations, including mutations in the WNT and RAS pathways (1). Despite advances in surgical techniques and adjuvant therapies, the survival rate of patients with colorectal cancer remains unsatisfactory. Local recurrence, metastasis, and drug resistance contribute to high mortality and poor survival outcomes (1, 2).

The serine-threonine kinase 11 (*STK11*) gene encodes the liver kinase B1 (LKB1) protein that functions as a tumor suppressor. STK11/LKB1 regulates various cellular processes, including cell growth, metabolism, energy maintenance, and survival, through interactions with p53 and AMP-activated protein kinase (AMPK) (3). Loss-of-function mutations in the *STK11* gene have been implicated in oncogenesis and may lead to tumorigenesis. Heterozygous germline mutations in the *STK11* gene are responsible for the development of hamartomas and gastrointestinal adenocarcinomas in patients with Peutz–Jeghers syndrome (PJS) (4). Inactivating somatic mutations in the *STK11* gene have been shown to promote tumor progression and metastasis (5, 6). STK11 mutation most frequently occurs in non-small cell lung cancer (NSCLC), melanoma, and cervical carcinoma (7). Additionally, STK11/LKB1 mutations often coexist with other gene variants such as those in *TP53* and *KRAS* in lung cancer (6). However, *STK11* mutations are rare in colorectal cancer, occurring in approximately 1.2% of cases (8).

Down-regulation of STK11/LKB1 activity or protein expression can be caused by loss-of-function mutations or epigenetic regulation, such as promoter hypermethylation or microRNA regulation (9). Studies have demonstrated that STK11/LKB1 deficiency is associated with tumor progression and invasion, as well as poor clinical outcomes in various cancer types, including NSCLC, breast cancer, hepatocellular carcinoma, and colorectal cancer (10-13). Moreover, STK11/LKB1 has been extensively studied in relation to the immunosuppressive tumor microenvironment in lung cancer models and NSCLC patients (14). In a mouse model, STK11/LKB1-deficient tumors presented reduced tumor-infiltrating lymphocyte (TIL) infiltration, lower PD-L1 expression, and increased exhausted T lymphocyte infiltration (15, 16). Several retrospective studies have also shown that STK11/LKB1 mutations in NSCLC patients are associated with resistance to anti-PD-1/PD-L1 immunotherapy, particularly in the presence of KRAS co-mutations (17, 18). The abundance of TILs in the tumor micro-environment, such as CD3+ and CD8+ TILs, is also a critical factor influencing survival outcomes in patients with colorectal cancer (19, 20). However, there has been limited research on the expression of STK11/LKB1 and its relationship with the tumor microenvironment in colorectal cancer.

To address this gap, we utilized immunohistochemistry (IHC) to assess the expression of STK11/LKB1 and the presence of various immune cell subsets, including TILs expressing CD45, CD45RO, CD8, and programmed cell

death protein 1 (PD-1). We evaluated the relationships between STK11/LKB1 expression, TILs, clinicopathological parameters, and survival outcomes in patients with colon adenocarcinoma (COAD). Our goal was to provide new insights into the potential of STK11/LKB1 expression to be used as a biomarker for the prognosis or a target for the treatment of COAD patients.

Materials and Methods

Study population. From 2012 to 2014, a total of 174 patients with colon adenocarcinoma (COAD) who underwent surgery, with or without postoperative chemotherapy, at China Medical University Hospital (CMUH) were recruited for this study. The study was approved by the Institutional Review Board (IRB) of CMUH (CMUH105-REC2-073). The resected samples were reviewed by pathologists, and pathological staging was performed according to the 7th edition of the American Joint Committee on Cancer (AJCC) staging system. Among the 174 patients, 169 had sufficient formalin-fixed, paraffin-embedded (FFPE) tissue samples available for tissue microarray (TMA) construction.

Tissue microarray (TMA) construction. TMAs were constructed from surgically resected normal mucosa and primary tumor FFPE tissues from COAD patients, as previously described (21). In brief, normal mucosa and tumor areas were marked on hematoxylin/eosin (HE)-stained tissue slides by a pathologist. The corresponding areas were then punched with 2-mm diameter tissue cylinders and transferred to recipient paraffin blocks for TMA construction. Each TMA spot included at least 50% tumor cells.

Immunohistochemical (IHC) staining and evaluation. IHC staining was performed on 3-μm-thick sections of TMAs as previously described (18). The antibodies that were used in this study were as follows: anti-human STK11/LKB1 (1:100, ab10538, Abcam, Cambridge, UK), anti-human CD45 (1:400, ab10538, Abcam), anti-human CD45RO (1:100, ab10538, Abcam), anti-human CD8 (1:400, ab4055, Abcam), and anti-human PD-1 (1:100, ab10538, Abcam) antibodies. Briefly, the antigens were retrieved with sodium citrate buffer, and the sections were incubated with primary antibody and then detected with an HRP-conjugated Vectastain Elite ABC Kit (Vector Laboratories, Newark, CA, USA) and NovaRed chromogen (Vector Laboratories) according to the manufacturer's protocol. Finally, the slides were counterstained with hematoxylin and mounted. STK11/LKB1 expression was assessed with a semiquantitative histoscore (H score). The intensity of STK11/LKB1 staining was categorized as follows: negative, weak, moderate, or strong. The H-score was calculated with the following formula: H-score =[1 × (% of weakly stained area) + 2 × (% of moderately stained area) + 3 × (% of strongly stained area)]. The H-score ranged from 0 to 300. Intratumor TILs were evaluated by counting the number of positively stained TILs at 400× magnification [number of positively stained TILs/high-power field (HPF)] as we previously described (18). The average number of TILs in five high-power fields was included in the evaluation and scored as follows: 0, a count of zero positively stained TILs/HPF; 1, a count of 1-3 positively stained TILs/HPF; 2, a count of 4-10 positively stained TILs/HPF; and 3, >10 positively stained TILs/HPF.

Statistical analysis. Statistical analysis was performed with JMP Pro software, version 12 (SAS Institute, Cary, NC, USA). A chi-square test was used to determine the correlation between STK11/LKB1 expression and clinicopathological characteristics. A p-value <0.05 was considered to indicate a statistically significant difference (two-sided). Kaplan–Meier analysis was used to assess overall survival (OS), and survival differences were analyzed with the log-rank test. The Cox proportional hazards model was used for multivariate analysis to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) and identify prognostic factors.

Results

Clinicopathological characteristics of patients with COAD. A total of 169 COAD patients were enrolled in this study, and their clinicopathological characteristics are summarized in Table I. The mean age at diagnosis was 62.8±12.8 years (range=31-92 years). The majority of patients were male (53%) and had well-differentiated or moderately differentiated histological grades (88%). All patients underwent surgery, and pathological staging was assessed according to the 7th edition of the American Joint Committee on Cancer (AJCC) staging system. Among these patients, the distribution of pT stages was as follows: 18 patients (11%) had stage 1 disease, 19 patients (11%) had stage 2 disease, 88 patients (52%) had stage 3 disease, and 44 patients (26%) had stage 4 disease. The distribution of pN stages was as follows: 84 patients (50%) had stage 0 disease, 45 patients (27%) had stage 1 disease, and 40 patients (23%) had stage 2 disease. Additionally, 76 patients (45%) presented with lymphovascular invasion (LVI), and 59 patients (35%) presented with perineural invasion (PNI). A total of 91 patients (54%) received adjuvant chemotherapy based on 5-fluorouracil and/or oxaliplatin following surgery. *KRAS* mutation testing was performed on 83 patients, and 36 patients (43%) were found to have *KRAS* mutations.

STK11/LKB1 expression and its relationship with clinicopathological characteristics. We evaluated STK11/LKB1 expression in paired normal mucosa and tumor tissues from COAD patients using IHC staining and H-scoring of TMAs. The STK11/LKB1 protein was primarily localized in the cytoplasm and exhibited varying expression (Figure 1). The distribution of STK11/LKB1 H-scores in the normal mucosa and tumors is shown in Figure 2, and the mean H-scores of STK11/LKB1 H-scores in the normal mucosa and tumors were 108.2 and 131.7, respectively. Notably, STK11/LKB1 expression was significantly greater in tumor tissues than in normal mucosa (Wilcoxon matched-pairs test, $p<0.0001$).

Table I. *Clinicopathological characteristics of patients with colorectal adenocarcinoma (n=169).*

Clinicopathological parameters	Cases (%)
Age	
<65	92 (54%)
≥65	77 (46%)
Sex	
Male	90 (53%)
Female	79 (47%)
Histological grade	
Well	22 (13%)
Moderate	124 (75%)
Poor	21 (12%)
pT stage	
1	18 (11%)
2	19 (11%)
3	88 (52%)
4	44 (26%)
pN	
0	84 (50%)
1	45 (27%)
2	40 (23%)
Lymphovascular invasion (LVI)	
Absent	92 (54%)
Present	76 (45%)
NA	1 (1%)
Perineural invasion (PNI)	
Absent	109 (64%)
Present	59 (35%)
NA	1 (1%)
Preoperative CEA (ng/ml)	
<5	88 (52%)
≥5	68 (40%)
NA	13 (8%)
Postoperative chemotherapy	
No	78 (46%)
Yes	91 (54%)
KRAS	
Wild type	47 (28%)
Mutant	36 (21%)
NA	86 (41%)

CEA: Carcinoembryonic antigen; NA: not available.

Next, we categorized tumor STK11/LKB1 expression as either low or high on the basis of the mean H-score and analyzed its association with clinicopathological parameters. As shown in Table II, we found that low STK11/LKB1 expression in tumors was significantly associated with pN stage ($p=0.0004$), but there was no significant correlation with other clinicopathological characteristics. Tumors with low STK11/LKB1 expression

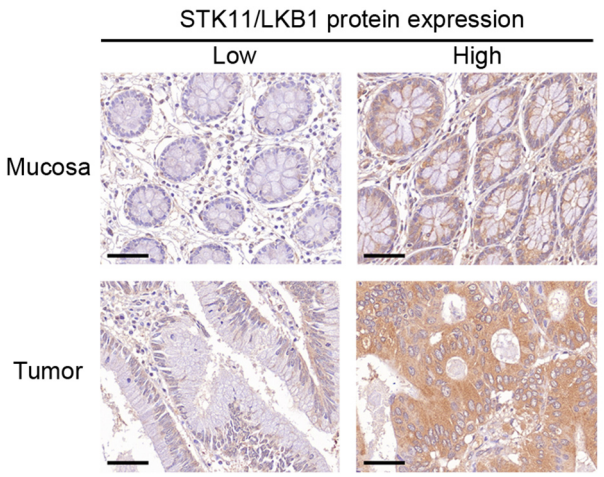

Figure 1. *Representative images of STK11/LKB1 immunohistochemical (IHC) staining from tissue microarrays of colorectal adenocarcinoma patients. IHC stain with STK11/LKB1 in the mucosa and tumor region. Scale bars=50 μm.*

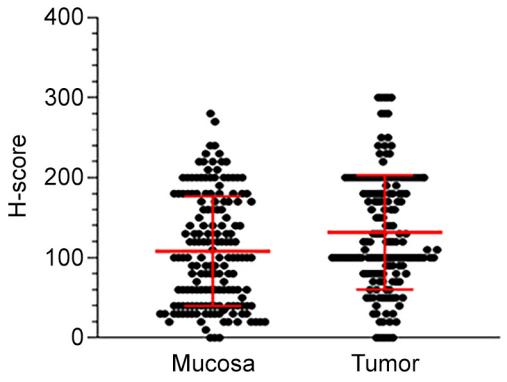

Figure 2. *STK11/LKB1 expression in the mucosa and tumor evaluated using the H-index. Data are represented as mean±SD. Wilcoxon matched-pairs test, ***p<0.001.*

Table II. *The relationship between clinicopathological parameters and STK11 expression in patients with colorectal adenocarcinoma.*

Clinicopathological parameters	STK11 expression		
	High 86 (100%)	Low 83 (100%)	*p*-Value
Sex			
Male	52 (60%)	38 (46%)	0.055
Female	34 (40%)	45(54%)	
Age			
<65	45 (52%)	47 (57%)	0.574
≥65	41 (48%)	36 (43%)	
Histological grading			
Well	12 (14%)	10 (12%)	0.894
Moderate	62 (74%)	62 (75%)	
Poor	10 (12%)	11 (13%)	
pT stage			
Early	23 (27%)	14 (17%)	0.119
Late	63 (73%)	83 (69%)	
pN			
Negative	52 (60%)	32 (39%)	0.004*
Positive	34 (40%)	51 (61%)	
Lymphovascular invasion (LVI)			
Absent	49 (57%)	43 (52%)	0.554
Present	37 (43%)	39 (48%)	
Perineural invasion (PNI)			
Absent	59 (69%)	50 (60%)	0.300
Present	27 (31%)	32 (39%)	
Preoperative CEA (ng/ml)			
<5	52 (63%)	36 (49%)	0.062
≥5	30 (37%)	38 (51%)	
NA	4	9	
KRAS			
Wild type	17 (49%)	30 (62%)	0.206
Mutant	18 (51%)	18 (38%)	
NA	51	35	

pN stage: negative (Stage 0) *vs*. positive (Stage 1a + 1b + 2); pT stage: early (Stage 1+2) *vs*. late (Stage 3+4); NA: not available. A chi-squared test was used. The contrast test did not include the "NA" group. *statistically significant.

were more likely to exhibit lymph node invasion. These findings suggest that STK11/LKB1 expression may be linked to nodal metastasis.

Relationship between tumor STK11/LKB1 expression and TILs. Down-regulation of STK11/LKB1 has been associated with an immunosuppressive tumor microenvironment and reduced immunotherapy efficacy in lung cancer. To explore the role of STK11/LKB1 expression in the tumor microenvironment of COAD, we evaluated the associations between STK11/LKB1 expression and intratumoral TILs with various immune cell markers. The markers employed for these immune cell subsets were as follows: CD45+ for all leukocytes, CD8+ for cytotoxic T cells, PD-1+ for activated T cells, and CD45RO+ for memory T cells. Then, we assessed the average number of TILs in high-power fields to determine their density grade. The density of CD45+, CD8+, PD-1+, and CD45RO+ TILs was then categorized as either

Table III. *The relationship between STK11 expression and tumor-infiltrating lymphocyte (TIL) density in patients with colorectal adenocarcinoma (n=169).*

TILs	STK11 expression		
	High (n=86)	Low (N=83)	*p*-Value
$CD45^+$ TIL density			
High	41 (48%)	38 (46%)	0.805
Low	45 (52%)	45 (54%)	
$CD8^+$ TIL density			
High	32 (37%)	23 (28%)	0.187
Low	54 (63%)	60 (72%)	
PD-1 TIL density			
High	18 (21%)	16 (19%)	0.788
Low	68 (79%)	67 (80%)	
CD45RO+ TIL density			
High	18 (21%)	16 (19%)	0.788
Low	68 (79%)	67 (81%)	

TIL: High (grade 2 + 3) *vs*. Low (grade 0 + 1); A chi-squared test was used.

low- (scores of 0 and 1) or high-grade (scores of 2 and 3). As shown in Table III, our data revealed no significant correlation between STK11/LKB1 expression and the density of CD45+ TILs or between STK11/LKB1 expression and the density of CD8+, PD-1+, or CD45RO+ TILs. These findings suggest that STK11/LKB1 expression may not contribute to the development of an immunosuppressive tumor microenvironment in COAD patients.

STK11/LKB1 expression is associated with survival outcomes. We performed Kaplan–Meier survival analysis to evaluate the impact of various parameters on OS in COAD patients. In this study, the OS rate of patients with COAD was 55%, and the median follow-up time was 38.2 months (2.2 to 67.8 months). As shown in Table IV, several clinicopathological characteristics, including histologic grade ($p<0.001$), pT stage ($p=0.002$), pN stage ($p<0.001$), lymphovascular invasion (LVI) ($p<0.001$), and perineural invasion (PNI) ($p=0.001$), were significantly associated with OS. Additionally, tumor STK11/LKB1 expression ($p=0.008$), PD-1+ TIL numbers ($p=0.046$), and CD45RO+ TIL numbers ($p=0.006$) were strongly correlated with OS. Patients with low expression of STK11/LKB1 had poorer OS (Figure 3A), as did those with low-grade PD-1+ and CD45RO+ TIL numbers. Hence, down-regulation of STK11/LKB1 in tumors and fewer activated T lymphocytes or memory T lymphocytes in the tumor microenvironment are

Table IV. *The relationship between clinicopathological parameters and overall survival in patients with colorectal adenocarcinoma.*

Clinicopathological parameters	Survival rate (%)	*p*-Value
Sex		
Male	56.0	0.844
Female	59.4	
Age		
<65	60.8	0.387
≥65	54.1	
Histological grading		
Well to moderate	28.6	<0.001*
Poor	62.2	
pT stage		
Early	81.1	0.002*
Late	50.8	
pN		
Negative	83.9	<0.001*
Positive	32.3	
Lymphovascular invasion (LVI)		
Absent	75.3	<0.001*
Present	36.7	
Perineural invasion (PNI)		
Absent	66.2	0.001*
Present	43.5	
KRAS		
Wild type	37.1	0.555
Mutant	27.7	
Tumor STK11 expression		
High	67.8	0.008*
Low	47.3	
$CD45^+$ TILs		
High	65.8	0.062
Low	50.6	
$CD8^+$ TILs		
High	68.7	0.128
Low	52.4	
$PD\text{-}1^+$ TILs		
High	72.6	0.046*
Low	53.9	
$CD45RO^+$ TILs		
High	72.7	0.006*
Low	48.3	

$CD3^+$ TILs: high (grade 2+3) *vs*. low (grade 0+1); TILs: Tumor-infiltrating lymphocytes. The Kaplan–Meier method was used for estimating survival probability, and the *p*-value was obtained from a log-rank test. *statistically significant.

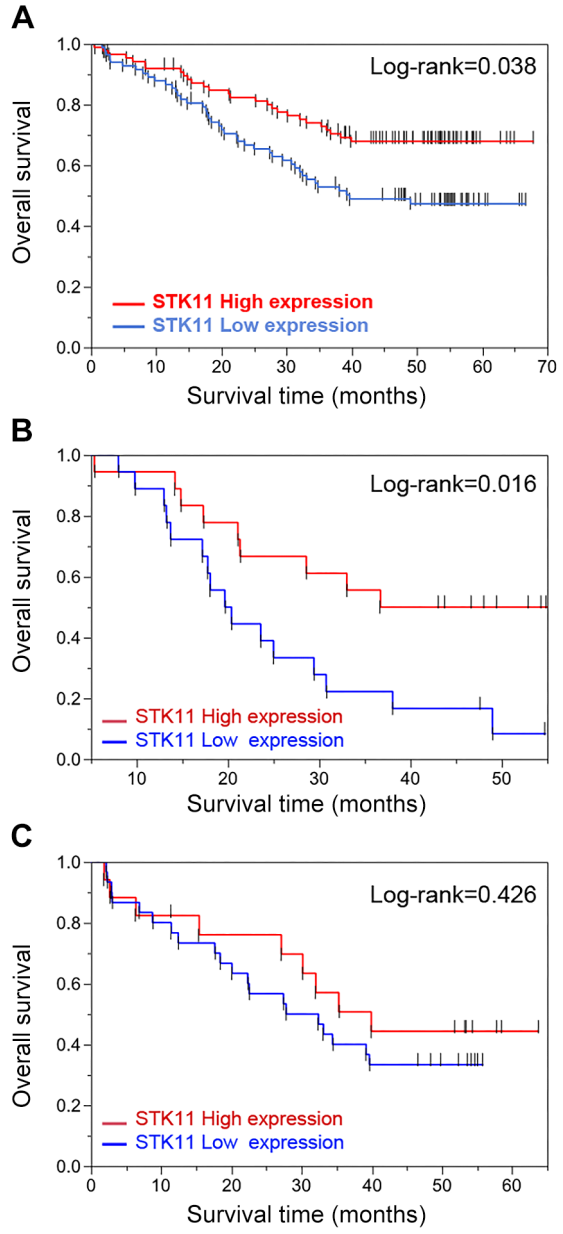

Figure 3. *The association between overall survival (OS) and STK11/LKB1 expression in colorectal adenocarcinoma (COAD) patients. The survival curves are presented by Kaplan–Meier survival analysis. (A) COAD patients, (B) COAD patients with KRAS mutation, (C) COAD patients with wild-type KRAS. The p-value was obtained from a log-rank test.*

associated with unfavorable survival outcomes in COAD patients. Notably, the association between STK11/LKB1 expression and OS was particularly pronounced in COAD patients with KRAS mutations compared with those with wild-type KRAS (Figure 3B and C). Specifically, patients with low STK11/LKB1 expression and KRAS mutations had significantly poorer survival outcomes.

We further utilized the Cox proportional hazards model in multivariate regression analysis and a forest plot to assess the prognostic value of STK11/LKB1 expression. As shown in Table V, we found that pN stage, LVI, and tumor STK11/LKB17 expression were independent risk factors for OS. Patients with high tumor STK11/LKB1 expression had a significantly increased risk of poor OS (HR=1.929, 95%CI=1.143-3.3171, p=0.014). Collectively, these results indicate that tumor STK11/LKB17 expression is an independent prognostic factor for COAD patients.

Discussion

STK11/LKB17 is a tumor suppressor gene. Germline or somatic mutations in the *STK11/LKB17* gene in tumors mostly cause truncation or loss of function of the STK11/LKB17 protein. Genomic alterations in this gene are common in NSCLC but rare in colorectal cancer (8). Epigenetic regulation can also contribute to STK11/LKB1 protein deficiency (9, 22). Hence, using the STK11 protein expression phenotype may be a better marker for assessing the roles and effects of STK11/LKB1 than its mutation status. In the present study, we used IHC staining and H-scores to evaluate the expression phenotype of STK11/LKB17 in COAD TMAs. We investigated the relationships between tumor STK11/LKB1 expression, clinicopathological characteristics, intratumor-infiltrating lymphocytes (TILs) and survival outcomes of patients with COAD. Our findings revealed that tumor STK11/LKB1 expression was strongly associated with nodal metastasis but did not correlate with other clinicopathological characteristics, such as pT stage, LVI, PNI, or KRAS mutation status. We also found no significant correlation between STK11/LKB1 expression and various TIL subsets, including CD45+, CD8+, PD-1+, and CD45RO+ TILs. Importantly, low STK11/LKB1 expression was

Table V. *Multivariate analysis of clinicopathological parameters and STK11 expression in relation to overall survival (n=162).*

	Multivariate		
	HR	95%CI	*p*-Value
Histological grading (poor *vs.* well to moderate)	1.558	0.829-2.771	0.161
pT stage (late *vs.* early)	1.175	0.459-3.433	0.746
pN stage (positive *vs.* negative)	3.482	1.632-7.915	0.001*
LVI (present *vs.* absent)	2.362	1.322-4.361	0.003*
PNI (present *vs.* absent)	0.922	0.542-1.577	0.766
Tumor STK11 expression (low *vs.* high)	1.929	1.143-3.317	0.014*
PD-1$^+$ TIL density (low *vs.* high)	1.48	0.709-3.406	0.307
CD45RO$^+$ TIL density (low *vs.* high)	1.209	0.661-2.325	0.546

TILs: Tumor-infiltrating lymphocytes. *statistically significant.

significantly associated with poor OS, suggesting that STK11/LKB1 expression may serve as an independent prognostic factor in COAD patients.

Previous research has demonstrated that the loss of STK11/LKB17 protein could promote tumor invasion, progression, metastasis, and poor survival outcomes in patients with solid tumors, particularly in patients with colorectal cancer, gastric cancer and NSCLC (13, 23, 24). For example, He *et al.* reported that the loss of STK11/LKB17 occurred *via* the NK2 homeobox 1 (NKX2-1)-mediated p53 pathway in colon cancer cell lines. They also reported that loss of STK11/LKB17 expression promoted tumor invasion and poor survival outcomes in patients with colorectal cancer (9). Chen *et al*. reported that knockdown of STK11/LKB17 induced tumor cell proliferation, migration, and invasion and was associated with the depth of invasion and lymph node metastasis in a mouse model (10). Sun *et al*. reported that decreased expression of STK11/LKB17 mRNA and protein was associated with shorter OS in gastric cancer patients (24). Consistent with previous studies, we found that low STK11/LKB1 expression was significantly associated with nodal metastasis and poor survival outcomes in COAD patients. Moreover, STK11/LKB17 mutations often co-occur with *KRAS* mutations and are associated with distant metastasis and poor outcomes in patients with NSCLC (6, 25, 26). We did not find a significant association between STK11/LKB1 expression and *KRAS* mutation in COAD, but low STK11/LKB1 expression was more common in *KRAS*-mutant COAD. Similar to patients with *KRAS*-mutant NSCLC, our data show those COAD patients with low STK11/LKB1 expression and *KRAS* mutations had significantly poorer survival outcomes than patients with wild-type *KRAS*.

STK11/LKB1 mutation or protein loss is a negative prognostic factor in NSCLC and influences cancer response to treatment, particularly to immunotherapy (14, 16, 27). Several studies have shown that STK11 mutations or deficiencies contribute to immunotherapy resistance in NSCLC patients and murine models. Retrospective studies observed that *STK11* mutation was associated with worse OS in NSCLC patients who receiving surgery and immune checkpoint inhibitors treatment (27). Mechanistic studies have revealed that STK11/LKB1 loss can decrease AMPK activity and increase S-adenosylmethionine, leading to attenuated antigen presentation and decreased stimulator of interferon genes (STING) and programmed death ligand 1 (PD-L1) expression in tumor cells (17). Consequently, the tumor microenvironment becomes immunosuppressive, with a lower number of TILs and inflammatory immune cells (15, 28). Therefore, the status of STK11/LKB1 is associated with the response to ICB immunotherapy. Moreover, the density and subset of TILs are not only important prognostic factors in patients with colorectal cancer but also predictive factors for the response to ICB immunotherapy. Patients with low TIL or CD8+ T cell

numbers have unsatisfactory responses to ICB immunotherapy (29, 30). Hence, we assessed the associations between tumor STK11/LKB1 expression and various intratumoral immune cell subsets, including CD45+ (pan leukocyte), CD8+ (cytotoxic T cells), PD-1+ (activated T cells), and CD45RO+ (memory T cells) TILs. However, we found no correlation between STK11/LKB1 expression and the proportions of these TIL subsets, indicating that STK11/LKB1 expression may not influence lymphocyte infiltration in COAD tumors or contribute to an immunosuppressive tumor microenvironment. Further clinical and animal studies are therefore needed to validate the present results.

STK11/LKB1 mutation analysis is limited in routine clinical practice, and IHC staining and H-scores provide a more comprehensive assessment of the STK11/LKB1 phenotype in tumors. Here, our study demonstrated that tumor STK11/LKB1 expression is an independent prognostic factor in COAD patients, regardless of clinicopathological parameters and TIL density. STK11/LKB1 expression has the potential to be a valuable prognostic biomarker in COAD patients and should be considered in clinical research and STK11-related cancer therapies.

Conflicts of Interest

The Authors have no conflicts of interest to report in relation to this study.

Authors' Contributions

Yung-Heng Lee, Chun-Fan Yang, Shu-Fen Chiang performed the experiments and the IHC evaluation; Kevin Chih-Yang Huang and K.S. Clifford Chao collected the clinical information of patients with COAD patients; Yih-Farng Liou and Shu-Fen Chiang performed the statistical analysis; Yung-Heng Lee, Chun-Fan Yang, Shu-Fen Chiang analyzed the data and wrote the manuscript; and Shu-Fen Chiang supervised this study and provided the funds. All Authors have read and approved the manuscript.

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