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ORIGINAL RESEARCH

Correlation Between DCAMKL-1 Protein Expression and K-ras Gene Mutation in Colorectal Cancer

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Aim: To investigate the correlation between doublecortin and CaM kinase-like-1 (DCAMKL-1) protein expression, K-ras gene mutation, and their impact on patient prognosis in colorectal cancer (CRC).

Methods: Immunohistochemistry was used to detect the expression of DCAMKL-1 protein in 60 cases of colorectal adenoma, 82 cases of CRC (including 65 cases of lymph node metastasis) and paraffin-embedded paracancerous intestinal mucosal tissue. K-ras gene mutations in primary CRC lesions were detected using an amplification-refractory mutation system and fluorescent polymerase chain reaction. The relationship between DCAMKL-1 protein expression and K-ras gene mutations with the clinicopathological characteristics of patients with CRC was analyzed. Univariate Kaplan–Meier survival analysis and multivariate Cox regression analysis were performed using follow-up data.

Results: The mutation rate of the K-ras gene in 82 cases of CRC was 48.8% (40/82). The positivity rate for the presence of DCAMKL-1 protein in CRC was 70.7% (58/82), significantly higher than that for colorectal adenomas (53.3%; 32/60) and paracancerous intestinal mucosa (0%; 0/82) ($P<0.05$). The positive expression rate for the presence of DCAMKL-1 protein in 65 patients with lymph node metastasis was higher in the primary lesions (69.2%; 45/65) than in the lymph node metastases (52.3%; 34/65) ($\chi^2=12.087$, $P=0.001$). The K-ras gene mutation status was positively correlated with DCAMKL-1 protein expression ($r=0.252$, $P=0.022$).

Conclusion: In this study, a potential positive correlation between K-ras gene mutation and DCAMKL-1 protein expression was identified in CRC tissues. The assessment of K-ras gene mutation status and DCAMKL-1 protein expression holds promise for augmenting early diagnosis and prognosis evaluation in CRC. This approach may improve the overall prognosis and survival outcomes for CRC patients.

Keywords: colorectal cancer, DCAMKL-1 protein, K-ras gene, mutation

Introduction

As one of the most common tumor entities worldwide, colorectal carcinoma accounts for approximately 10% of all tumor-related deaths.[1] With improvements in living standards, changes in dietary habits, and aging of the population structure, the incidence of CRC is increasing year by year.[2] Despite current considerable advances in CRC screening and diagnosis, patient survival times remain short.[2] While recent years have witnessed significant advancements in colorectal cancer treatment through the introduction of novel chemotherapy drugs and protocols, a substantial portion of patients experience limited efficacy or even relapse post-treatment.[3] This can be attributed to the inherent or acquired drug resistance mechanisms associated with anti-tumor drugs.[4] The development of CRC is a multistep process involving multiple genes and factors; for example, both the activation of K-ras oncogenes and the mutation and deletion of *TP53* oncogenes are involved in this process.[5] K-ras gene mutations are common adverse events in CRC; the mutation rate in Asian patients ranges from 29% to 62.9%.[6,7] Patients with K-ras gene mutations are more prone to recurrence, metastasis, and drug resistance, and K-ras gene mutations are recognized biomarkers that can be used to guide the clinical treatment of CRC.[8]

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Cancer stem cells (CSCs) have strong self-renewal and proliferation capabilities and are involved in the formation, recurrence, and drug resistance of CRC. In recent years, doublecortin and CaM kinase-like-1 (DCAMKL-1), a transmembrane microtubule-related kinase found in postmitotic neurons, has been found in various solid tumors, such as CRC and pancreatic cancer;[9] additionally, it is also considered to be an intestinal stem cell marker. Currently, there is limited data on the expression of DCAMKL-1 protein in human CRC tissue, and the expression of DCAMKL-1 during progressive tumorigenesis has rarely been studied. In addition, there are few literature reports on whether there is a correlation between K-ras gene mutations and DCAMKL-1 protein expression in CRC tissue.

To explore these questions, this study detected the expression of DCAMKL-1 protein in colorectal adenoma, CRC, paracancerous intestinal mucosa, and lymph node metastasis specimens by immunohistochemistry. DCAMKL-1 protein expression differences among the 4 different tissues and its relationship with the clinicopathological characteristics of CRC patients were investigated to preliminarily explore the role of DCAMKL-1 protein in the occurrence and development of CRC and its relationship with the prognosis of CRC patients. Second, the mutation status of the K-ras gene in primary CRC lesions was detected using an amplification-refractory mutation system (ARMS) and fluorescence PCR, and its clinical significance and correlation with DCAMKL-1 protein expression were preliminarily investigated.

Materials and Methods

Clinical Data

Endoscopic biopsies/sectioned specimens of 60 cases of colorectal adenomas, 82 cases of CRC primary lesions (54 males and 28 females; age 32–89 years and median age of 59 years), paracancerous intestinal mucosa specimens and corresponding paraffin-embedded tissue specimens from 65 cases of lymph node metastases between December 2014 and April 2019 were retrospectively obtained from Guizhou Provincial People's Hospital. All specimens were diagnosed by 2 pathologists separately and evaluated for the degree of differentiation of intestinal cancer (10 cases of high differentiation, 54 cases of moderate differentiation, and 18 cases of low differentiation). Clinical staging was performed in accordance with the seventh edition of the American Joint Committee on Cancer (AJCC) guidelines (2 cases of stage I, 10 cases of stage II, 33 cases of stage III, and 37 cases of stage IV).

This study was adhered to the principles of the Declaration of Helsinki and approved by the Ethics Committee of Guizhou Provincial People's Hospital (approval number: 2019-63), which exempted the requirement for written informed consent due to the retrospective nature of the data collection and the use of deidentified clinical data.

Inclusion and Exclusion Criteria for CRC

The inclusion criteria were as follows: (1) CRC confirmed by postoperative pathological examination; (2) no chemoradiotherapy or biological treatment performed before surgery; and (3) complete clinical and pathological data.

The exclusion criteria were as follows: (1) in addition to CRC, a history of other organ tumors; (2) family history of CRC in first- or second-degree relatives; and (3) serious cardiovascular disease.

Main Reagents

Currently, the most commonly used methods for detecting K-ras gene mutations in paraffin-embedded tissues are amplification and refractory mutation system (ARMS) and fluorescent PCR. These methods are more cost-effective compared to the first and second-generation sequencing techniques, making them more suitable for implementation in medical institutions. Immunohistochemistry is widely regarded as the most direct and commonly employed method for detecting protein expression. It can be conducted in nearly all laboratories across medical institutions of varying levels. Therefore, in this study, ARMS along with fluorescent PCR were employed to detect K-ras gene mutations, while immunohistochemistry was utilized to assess DCAMKL-1 protein expression. The paraffin-embedded tissue nucleic acid extraction kit and human K-ras gene mutation detection kit (utilizing the fluorescent PCR method) were procured from Xiamen Amoy Biomedical Technology Co. Ltd. For immunohistochemical staining, a concentrated rabbit anti-human DCAMKL-1 polyclonal antibody (clone number ab31704, Abcam, USA), an SP staining kit, and a DAB staining kit were obtained from Beijing Zhongshan

Golden Bridge Biological Co., Ltd. The immunohistochemical SP method kit and DCAMKL-1 antibody utilized in this study were of standard quality.

Experimental Method

DNA Extraction and Gene Mutation Detection

CRC surgical resection specimens (fixed in 10% neutral formalin solution and embedded in paraffin) were serially sectioned (4 μm), and 3 to 5 slices were taken from each case and placed into EP tubes for DNA extraction; the tumor cell content in each slice was >60%. DNA extraction was performed according to the instructions provided with the paraffin-embedded tissue nucleic acid extraction kit. The DNA concentration was determined using a Nanodrop 2000, and the OD260/OD280 of the extracted DNA was between 1.8 and 2.0. Sample preparation and fluorescence quantitative PCR amplification were performed according to the instructions provided with the human K-ras gene mutation detection kit (fluorescence PCR method). The kit tested for 12 somatic hot spot mutations on exons 2, 3, and 4 of the K-ras gene.

Immunohistochemistry

Immunohistochemistry was performed according to the instructions provided with the immunohistochemistry SP method kit. The DCAMKL-1 antibody was diluted 1:100. The samples were subjected to high-pressure heat-induced antigen retrieval before incubation with the antibody. Cancer tissue with known positive expression was used as a positive control, and phosphate-buffered saline (PBS) was used instead of primary antibody as a blank control.

Interpretation of the Results

All results were interpreted by 2 senior pathologists. The interpretation of the K-ras gene results was carried out following the method described in the instructions provided with the human K-ras gene mutation detection kit. The DCAMKL-1 results were interpreted following the method described by Gao et al.[10] DCAMKL-1 protein localized in the cytoplasm was evaluated by combining 2 independent scoring factors, ie, staining intensity (0=none, 1=slight, 2=mild, 3=strong) and staining area 0 (<5%), 1 (5~25%), 2 (26~50%), 3 (51~75%) and 4 (>75%); the 2 scores were multiplied, resulting in possible scores of 0, 1, 2, 3, 4, 6, 8, 9, and 12. DCAMKL-1 immunostaining was considered positive when the value was equal to or greater than 4, and immunostaining was considered negative when the value was 0 to 3.

Statistical Methods

Statistical analysis was performed using SPSS 25.0 statistical software. The χ^2 test was used to compare the differences in expression between different groups. Spearman rank correlation analysis was performed. The Kaplan–Meier survival curve method, Log rank test, and Cox regression analysis were used for the survival analysis. $P<0.05$ was considered statistically significant.

Results

Data Characteristics

The study subjects were characterized based on their age, sex, tumor differentiation, clinical stage, and lymph node metastasis. A total of 60 colorectal adenomas were included in the study, with an average age of 57.82 years (ranging from 38 to 81 years), and 30% of the subjects were female. Among the 82 patients diagnosed with colorectal cancer, the mean age was 58.46 years (ranging from 32 to 89 years), with 34.1% being female. Tumor differentiation analysis revealed that 12.2% of cases were highly differentiated, 65.9% were moderately differentiated, and 21.9% were poorly differentiated. The clinical stages based on AJCC classification were as follows: stage I (2.5%), stage II (12.2%), stage III (40.2%), and stage IV (45.1%). The clinical stages based on AJCC classification were as follows: stage I (2.4%), stage II (12.2%), stage III (40.2%), and stage IV (45.1%). Notably, 79.3% of patients exhibited lymph node metastasis.

The Relationship Between K-ras Gene Mutations and the Clinicopathological Characteristics of CRC Patients

In this study, the K-ras gene in 82 CRC specimens was analyzed. The results showed that the K-ras gene was mutated in 40 cases (48.8%) and not mutated (wild type) in 42 cases (51.2%). The relationship between the K-ras gene mutation status in CRC tissue and various clinicopathological characteristics was analyzed. The results showed that the K-ras gene mutation status was not associated with patient age, sex, clinical stage, degree of tumor differentiation, or lymph node metastasis (P>0.05) (Table 1).

The Relationship Between DCAMKL-1 Protein Expression and the Clinicopathological Characteristics of CRC Patients

χ^2 analysis showed that DCAMKL-1 protein expression was not related to patient age, sex, clinical stage, degree of tumor differentiation, or lymph node metastasis (all P>0.05). (Table 1).

DCAMKL-1 Protein Expression in Colorectal Adenoma, CRC, and Paracancerous Intestinal Mucosal Tissues

DCAMKL-1 in colorectal adenoma and CRC tissues was mainly located in the cytoplasm. In the paracancerous intestinal mucosa tissue, there were only a few individual cells in glandular crypts with cytoplasmic staining, deemed negative according to the interpretation standard for DCAMKL-1. DCAMKL-1 protein expression was greatest in CRC tissue, followed by colorectal adenoma tissue and paracancerous intestinal mucosa tissue (χ^2=91.242, P=0.000). The positive expression rate of DCAMKL-1 protein in CRC tissue (58/82, 70.7%) was significantly higher than that in colorectal adenoma tissue (32/60, 53.3%) and paracancerous intestinal mucosa tissue (0/82, 0%) (χ^2=4.519, P=0.034; χ^2=89.736, P=0.000) (Table 2, Figure 1A–E).

Expression of DCAMKL-1 Protein in Primary CRC Lesions and Lymph Node Metastases

The positive expression rate of DCAMKL-1 protein was higher in primary lesions (69.2%, 45/65) than in lymph node metastases (52.3%, 34/65) (χ^2=12.087, P=0.001) (Figure 1C and F). Spearman correlation analysis indicated that the

Table 1 The Relationships of K-Ras Gene Mutation Status and DCAMKL-1 Protein Expression with the Clinicopathological Characteristics of CRC Patients

Clinicopathological Characteristics	Case Number	K-ras Gene		χ^2	*P*	DCAMKL-1		χ^2	*P
		Mutant	Wild			Positive	Negative		
Age (years)									
≤65	61	30	31	0.015	0.902	44	17	0.225	0.635
>65	21	10	11			14	7		
Sex									
Male	54	24	30	1.190	0.275	35	19	0.023	0.880
Female	28	16	12			23	5		
Clinical stage									
I+II	12	7	5	0.513	0.474	9	3	0.000	0.993
III+IV	70	33	37			49	21		
Degree of tumor differentiation									
High and moderate	64	33	31	0.903	0.342	46	18	0.184	0.668
Low	18	7	11			12	6		
Lymph node metastasis									
Yes	65	32	33	0.025	0.873	45	20	0.081	0.776
No	17	8	9			13	4		

Notes: *P* value was calculated using the χ^2 test between the mutant and wild-type K-ras genes. **P* value was calculated using χ^2 test between positive and negative DCAMKL-1 protein expression.
Abbreviation: CRC, colorectal cancer.

Table 2 Expression of DCAMKL-1 Protein in Different Colorectal Tissues

Tissue Type	Case Number	DCAMKL-1		χ^2	*P*
		Positive	Negative		
Paracancerous intestinal mucosa	82	0	82	89.736	*P=0.000[a]
Colorectal adenoma	60	32	28	56.456	**P=0.000[a]
CRC	82	58	24	4.519	***P=0.034[a]

Notes: [a]$P<0.05$. *P value was calculated using the χ^2 test between CRC and paracancerous intestinal mucosa. **P value was calculated using the χ^2 test between colorectal adenoma and paracancerous intestinal mucosa. ***P value was calculated using the χ^2 test between CRC and colorectal adenoma.
Abbreviation: CRC, colorectal cancer.

expression of DCAMKL-1 protein in primary lesions was positively correlated with lymph node metastasis (r=0.431, P=0.000) (Table 3).

Correlation Between K-Ras Gene Mutations and DCAMKL-1 Protein Expression in CRC Tissue

For the 40 cases of CRC in which the K-ras gene was mutated, DCAMKL-1 protein expression was observed in 33 cases, for a positive expression rate of 82.5% (33/40). For the 42 cases of CRC in which the wild-type K-ras gene was present, DCAMKL-1 protein expression was observed in 25 cases, for a positive rate of 59.5% (25/42). K-ras gene mutation was positively correlated with DCAMKL-1 protein expression (r=0. 252, P=0.022) (Table 4).

The Relationship Between K-ras Gene Status and DCAMKL-1 Protein Expression and the Survival and Prognosis of CRC Patients

In this study, we employed a combination of outpatient reviews, inpatient examinations, and telephone calls for postoperative follow-up. Follow-up ended in December 2021, and there were follow-up data for 66 patients. The follow-

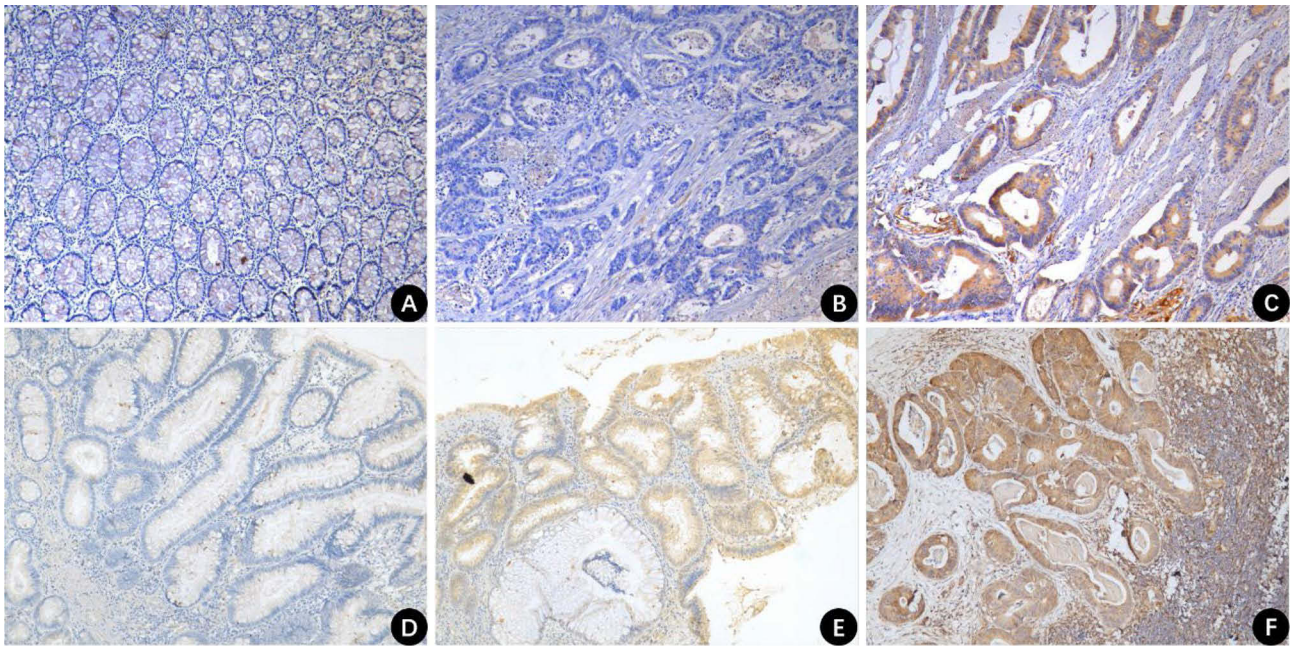

Figure 1 Expression of DCAMKL-1 protein in colorectal adenoma, CRC, paracancerous intestinal mucosa, and lymph node metastasis tissues (100× magnification). (**A**) negative DCAMKL-1 expression in paracancerous intestinal mucosal tissue; (**B**) negative DCAMKL-1 expression in CRC tissue; (**C**) positive DCAMKL-1 expression in CRC tissue; (**D**) negative DCAMKL-1 expression in colorectal adenoma; (**E**) positive DCAMKL-1 expression in colorectal adenoma; (**F**) positive DCAMKL-1 expression in lymph node metastases.

Table 3 Expression and Correlation of DCAMKL-1 Protein Expression in Primary CRC Lesions and Lymph Node Metastases

Tissue Type	Case Number	DCAMKL-1		χ^2	*P1*	*r*	*P2*
		Positive	Negative				
Primary CRC lesions	65	45	20	12.087	0.001[a]	0.431	0.000[a]
Lymph node metastasis	65	34	31				

Notes: [a]*P*<0.05. *P1* value was calculated using the χ^2 test between primary CRC lesions and lymph node metastases. r and *P2* represent the correlation coefficient and P value of DCAMKL-1 protein expression, respectively, between primary CRC lesions and lymph node metastases (Spearman rank correlation).
Abbreviation: CRC, colorectal cancer.

Table 4 Correlation Between K-Ras Gene Mutation Status and DCAMKL-1 Protein Expression

K-ras	Case Number	DCAMKL-1		*r*	*P*
		Positive	Negative		
Mutant	40	33	7	0.252	0.022[a]
Wild	42	25	17		

Notes: [a]*P*<0.05. r and *P* represent the correlation coefficient and P value, respectively, of K-ras gene mutation status and DCAMKL-1 protein expression (Spearman rank correlation).

up rate was 80.5% (66/82), and the follow-up time ranged from 6 to 84 months. Twenty-six patients (39.4%) died, and 40 (60.6%) survived. Kaplan–Meier survival analysis showed that the 1-year survival rate was 86.4%, the 2-year survival rate was 78.8%, the 3-year survival rate was 67.8%, and the 5-year survival rate was 54.5%. Sex, age (≤65 years old group and >65 years old group), clinical stage (I+II group and III+IV group), degree of tumor differentiation (low differentiation group and high/moderate differentiation group), lymph node metastasis (yes and no), postoperative chemotherapy (yes and no), K-ras gene (mutant and wild type) and DCAMKL-1 protein expression (negative and positive) were subjected to univariate Kaplan–Meier survival analysis. The results indicated that clinical stage, degree of tumor differentiation, and K-ras gene status were significantly correlated with prognosis (Table 5). The prognosis of patients with stage I+II disease was significantly better than that of those with stage III+IV disease (χ^2=6.133, P=0.013) (Figure 2A). The prognosis of patients in the high/moderate differentiation group was significantly better than that of those in the low differentiation group (χ^2=14.507, P=0.000) (Figure 2B). Although there was no significant difference in the overall survival rate between the group with lymph node metastasis and the group without lymph node metastasis (χ^2=3.741, P=0.053), a trend was observed (Figure 2C). Patients with the wild-type K-ras gene had a significantly better

Table 5 Univariate and Multivariate Cox Regression Analyses of CRC Patients

Factor	Univariate Analysis			Multivariate Analysis		
	RR	95% CI	*P*	RR	95% CI	*P*
Age (≤65 years old and >65 years old)	1.000	0.965~1.036	0.980			
Sex (male and female)	0.903	0.392~2.081	0.811			
Clinical stage (I+II and III+IV)	2.600	1.341~5.041	0.005[a]	2.717	1.331~5.548	0.006[a]
Low and high/moderate differentiation	0.241	0.108~0.535	0.000[a]	0.253	0.112~0.572	0.001[a]
Lymph node metastasis (yes and no)	3.745	0.884~15.856	0.073			
Postoperative chemotherapy (yes and no)	1.520	0.456~5.069	0.496			
K-ras gene (mutant and wild)	0.366	0.163~0.826	0.015[a]	0.429	0.187~0.984	0.046[a]
DCAMKL-1 expression (negative and positive)	1.317	0.552~3.140	0.535			

Note: [a]*P*<0.05.
Abbreviations: RR, relative risk; CI, confidence interval; CRC, colorectal cancer.

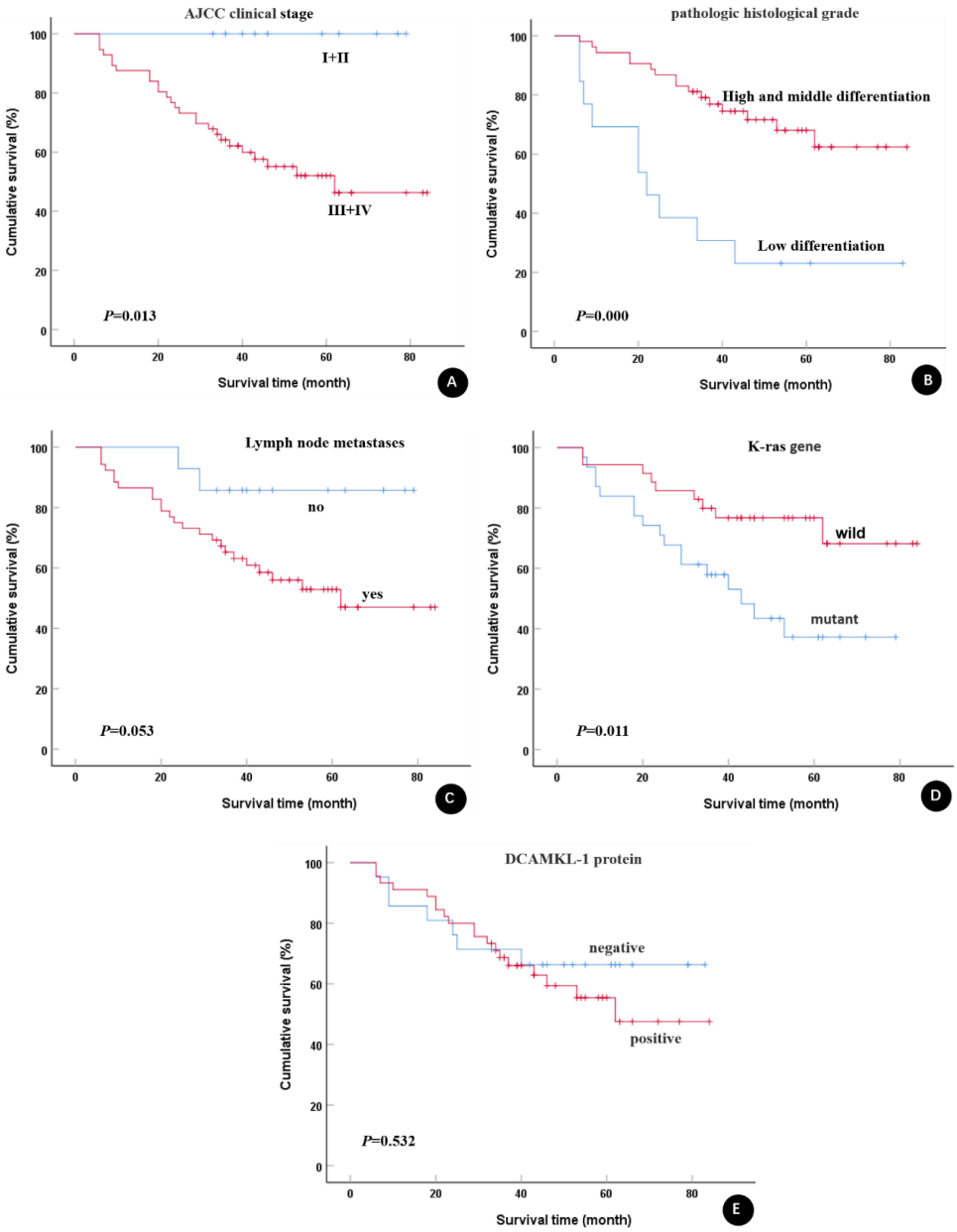

Figure 2 The relationship between clinicopathological parameters, K-ras gene status, and DCAMKL-1 protein expression and the survival time of CRC patients. (**A**) the effect of the clinical stage on the survival time of CRC patients; (**B**) the effect of degree of tumor differentiation on the survival time of CRC patients; (**C**) the effect of lymph node metastasis on the survival time of CRC patients; (**D**) the effect of K-ras gene status on the survival time of CRC patients; (**E**) the effect of DCAMKL-1 protein expression on the survival time of CRC patients.

prognosis than those with a mutated K-ras gene (χ^2=6.418, P=0.011) (Figure 2D). DCAMKL-1 protein expression was not significantly correlated with patient prognosis (P>0.05) (Figure 2E). Multivariate Cox analysis indicated that clinical stage, degree of tumor differentiation, and K-ras gene status were independent factors affecting the postoperative prognosis of CRC patients (RR=2.717, P=0.006; RR=0.253, P=0.001; RR=0.429, P=0.046) (Table 5).

Discussion

The K-ras gene is a member of the proto-oncogene rat sarcoma protein family. It is an important initiator and promoting factor in the occurrence and development of CRC. Patients with K-ras gene mutations are more prone to recurrence, metastasis, and drug resistance than are patients with the wild-type K-ras gene.[11–13] K-ras is a common oncogene in CRC. The mutation rate of the K-ras gene in this study was 48.8% (40/82), a finding that is consistent with the mutation rate of 29–62.9% reported in

the literature.[6,7] In this study, K-ras gene mutation status was not associated with age, sex, clinical stage, degree of tumor differentiation, or lymph node metastasis, findings that are consistent with the literature. Ines et al[14] also did not find a correlation between K-ras gene mutation status and clinicopathological features of CRC patients. However, Malhotra et al[15] found that K-ras gene mutation status was associated with patient age and tumor tissue differentiation. The reasons for the above differences may be related to various factors, such as sample size, sample population, patient lifestyle, and different genetic backgrounds, and these differences need to be further investigated using large sample data.

The human DCAMKL-1 gene is located on the long arm of chromosome 13 (13q13), and DCAMKL-1 protein is a protein kinase found in postmitotic neurons. Recent studies have found that DCAMKL-1 is a CSC marker that exists in various solid tumors, such as pancreatic cancer, colon cancer, hepatocellular carcinoma, and prostate cancer.[16–18] In this study, the expression of DCAMKL-1 protein was found to be minimal in surrounding noncancerous intestinal mucosa tissue, with only a small number of glandular crypts exhibiting cytoplasmic staining in some cells. Conversely, cytoplasmic overexpression of DCAMKL-1 protein was observed in CRC tissue, with 70.7% (58 out of 82) of cases showing significant overexpression. This level of overexpression was significantly higher compared to colorectal adenomas (53.3%, 32 out of 60) and surrounding noncancerous intestinal mucosa tissue (0%, 0 out of 82). Notably, similar findings were reported by Sureban et al[19] who demonstrated the significant overexpression of DCAMKL-1 protein in CRC tissue when compared to normal intestinal mucosa. The results of our study indicate a strong association between DCAMKL-1 protein expression and the development of colorectal cancer (CRC). This suggests that detecting DCAMKL-1 protein expression could serve as a valuable tool for early CRC diagnosis. Additionally, targeting DCAMKL-1 protein could potentially impede the progression of colorectal adenoma to adenocarcinoma. Nakanishi et al[9] found that in experimental mice with CRC, DCAMKL-1 protein-positive cells developed into tumor cells. This research group targeted and eliminated DCAMKL-1 protein-positive cells through genetic manipulation, and the CRC tissue area in the experimental mice decreased more than 80% and, in some, completely disappeared, without damage to the normal intestine. This research group suggested that targeting DCAMKL-1 has profound inhibitory effects on CSCs and can be used as a strategy to develop anticancer drugs with few side effects. Kim et al[20] also proposed the possibility of clinical trials of DCAMKL-1-targeted inhibitors for cancer treatment.

In this study, DCAMKL-1 protein expression was not associated with age, sex, clinical stage, degree of tumor differentiation, or lymph node metastasis. Harada et al[21] assessed (immunohistochemistry) DCAMKL-1 protein expression in surgical specimens from 106 patients with rectal cancer (including those who received preoperative chemoradiotherapy) and found that in patients with rectal cancer who received preoperative chemoradiotherapy, DCAMKL-1 expression and lymph node metastases were significantly associated, whereas no such association was found in patients who did not receive preoperative chemoradiotherapy. Gagliardi et al[22] found no relationship between DCAMKL-1 expression and clinicopathological features in CRC patients who did not receive preoperative chemoradiotherapy, similar to the results of this study. The reason for this difference between preoperative chemoradiotherapy and no chemoradiotherapy in patients with CRC requires further investigation.

In this study, DCAMKL-1 protein expression in primary CRC lesions (69.2%, 45/65) was higher than that in lymph node metastases (52.3%, 34/65). In contrast, Gao et al[10] showed that DCAMKL-1 protein expression in primary lesions was lower than that in lymph node metastases. The differences in the results of the studies may be related to the selection of lymph node metastases and the sample sizes. Currently, there are no reports on the correlation between primary CRC lesions and the corresponding lymph node metastases in the same patient. In our study, there was a positive correlation between DCAMKL-1 protein expression in primary CRC lesions and lymph node metastases, suggesting that this correlation may be related to tumor evolution. It is speculated that the 2 can be used as indicators of each other. In particular, when surgeons evaluate patients with advanced CRC who have no opportunity for surgery, biopsies of highly suspected lymph node metastases can be used to assess DCAMKL-1 protein expression in the primary tumor.

K-ras gene mutations can upregulate the downstream ERK pathway, thereby activating Wnt/β-catenin pathway signal transduction and then upregulating the expression of CSC markers in CRC cells.[23] As a newly discovered CSC marker, there are currently only a few reports in the literature about the relationship between K-ras gene mutations and DCAMKL-1 protein expression. Using a variety of K-ras mutant pancreatic cancer mouse models, Bailey et al[24] found that DCAMKL-1 is closely related to K-ras mutant pancreatic cancer. In addition, Westphalen et al[25] reported that K-ras-mutated DCAMKL-1-positive cells initiate the cancer program during pancreatic inflammation when K-ras forms a complex with DCAMKL-1. At the

molecular level, studies using the wild-type K-ras CRC cell line SW48 showed that when K-ras-G12D, G12V, or D13D was introduced for transcriptional induction, DCAMKL-1 was significantly upregulated; a dose-dependent decrease in DCAMKL-1 expression was observed when shRNA targeted the mutated K-ras gene in SW48 cells.[26] Similarly, when siRNA targeted DCAMKL-1, the expression of K-ras decreased in a dose-dependent manner.[27,28] Qu et al[29] found that DCAMKL-1 can activate the K-ras gene through the K-ras-PI3K-MTOR signaling pathway in pancreatic cancer. These studies were all carried out based on cytology. This is the first study to conduct a correlation analysis at the histological level. The results of this study showed that the positive rate of DCAMKL-1 expression in the K-ras gene mutation group (82.5%, 33/40) was higher than that in the wild-type K-ras group (59.5%, 25/42). K-ras gene mutation was positively correlated with DCAMKL-1 protein expression (r=0.252, P=0.022). In CRC tissue, immunopositive DCAMKL-1 protein expression might predict the expression of K-ras mutant genes and might be able to be used as an indirect indicator of K-ras gene status.

In this study, the follow-up data of 66 patients with CRC were used for univariate survival analysis. The survival rate of patients with clinical stage III+IV disease was significantly lower than that of patients with stage I+II disease, and the survival rate of patients with low differentiated tumors was significantly lower than that of patients with highly/moderately differentiated tumors. The survival rate of patients with K-ras mutants was significantly lower than that of patients with wild-type K-ras. These results suggest that advanced clinical stage, low tumor differentiation, and K-ras gene mutation are factors for a poor prognosis of CRC patients. There is some controversy about the correlation between K-ras gene mutations and the prognosis of CRC patients. The results of a Phase II clinical trial with a large sample size (4268 cases) showed that in patients with Dukes C stage CRC, the overall survival time and progression-free survival were shorter for patients with the K-ras gene exon 2 codon 12 mutation that for patients without mutations; the difference was statistically significant.[30] The results of several studies also showed that compared with that of CRC patients with the wild-type K-ras gene, the prognosis of patients with K-ras gene mutations was worse; the difference was statistically significant.[31–33] In this study, the survival rate for patients with K-ras mutations was significantly lower than that for patients with wild-type K-ras, a finding that is consistent with literature reports. However, some studies have also shown that K-ras gene mutations are not significantly correlated with the prognosis of CRC patients.[34] This study did not find a correlation between DCAMKL-1 protein expression and patient survival time, a finding that is not consistent with the results of a study by Gagliardi et al[22] who found that DCAMKL-1 expression was associated with a poor prognosis for CRC patients. However, there are also reports in the literature that the expression of DCAMKL-1 in rectal cancer patients treated with preoperative chemoradiotherapy was significantly correlated with prognosis, but no correlation was found in patients who did not receive preoperative treatment, findings that are consistent with those of this study.[21] The reason for this difference between preoperative chemoradiotherapy and no chemoradiotherapy in CRC patients requires further investigation. Further multivariate survival analysis revealed that AJCC clinical stage, tumor differentiation degree, and K-ras gene status independently influence the prognosis of CRC patients. This suggests that these three factors bear substantial clinical relevance in the prognostic assessment of CRC patients and can be employed more effectively in the clinical evaluation of patient outcomes.

There are several limitations in this study. First, the sample size used in this study is relatively small. The restricted availability of participants meeting the research criteria constrained our ability to gather a larger sample. As a result, it may impact the overall generalizability of our findings. In addition, the retrospective nature of data collection in this study may have introduced a certain level of selection bias. We will continue to increase the sample size and perform a prospective validation study to further strengthen the reliability and generalizability of our findings in the near future.

Conclusion

In this study, we confirmed that K-ras gene mutations and DCAMKL-1 protein expression are involved in the occurrence and development of CRC. This is the first study to identify a positive correlation between K-ras gene mutations and DCAMKL-1 protein expression in CRC tissue. While the correlation is not highly robust, there is a distinct tendency for mutated tissues to exhibit higher levels of the protein. This suggests that DCAMKL-1 protein expression could potentially serve as an indirect indicator for clinically predicting K-ras gene status. We plan to further validate this result in a prospective validation study in the future. In addition, we confirmed that advanced clinical stage, low tumor differentiation, and K-ras gene mutations were independent risk factors for a poor prognosis. This study provides

a theoretical basis for the selection of postoperative treatment options, prognostic assessments, and follow-up strategies for CRC patients.

Data Sharing Statement

The main data supporting the results of the study are available in the manuscript. The raw datasets cannot be made available due to hospital regulation restrictions and patient privacy concerns. Some anonymized data may be available for research purposes from the corresponding authors upon reasonable request.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflict of interest.

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