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Contents

Weekly Volume 26 Number 43 November 21, 2020

OPINION REVIEW

6706 Gastric acid level of humans must decrease in the future

Fuiimori S

EXPERT CONSENSUS

6710 First United Arab Emirates consensus on diagnosis and management of inflammatory bowel diseases: A 2020 Delphi consensus

Alkhatry M, Al-Rifai A, Annese V, Georgopoulos F, Jazzar AN, Khassouan AM, Koutoubi Z, Nathwani R, Taha MS, Limdi JK

MINIREVIEWS

6770 Hepatocellular carcinoma after direct-acting antiviral hepatitis C virus therapy: A debate near the end Muzica CM, Stanciu C, Huiban L, Singeap AM, Sfarti C, Zenovia S, Cojocariu C, Trifan A

ORIGINAL ARTICLE

Basic Study

6782 Lactobacillus bulgaricus inhibits colitis-associated cancer via a negative regulation of intestinal inflammation in azoxymethane/dextran sodium sulfate model

Silveira DSC, Veronez LC, Lopes-Júnior LC, Anatriello E, Brunaldi MO, Pereira-da-Silva G

6795 Antifungal activity and antidiarrheal activity via antimotility mechanisms of (-)-fenchone in experimental models

Pessoa MLS, Silva LMO, Araruna MEC, Serafim CAL, Alves Júnior EB, Silva AO, Pessoa MMB, Diniz Neto H, de Oliveira Lima E, Batista LM

- 6810 Effects of Yue-Bi-Tang on water metabolism in severe acute pancreatitis rats with acute lung-kidney injury Hu J, Zhang YM, Miao YF, Zhu L, Yi XL, Chen H, Yang XJ, Wan MH, Tang WF
- 6822 Tissue microarray-chip featuring computerized immunophenotypical characterization more accurately subtypes ampullary adenocarcinoma than routine histology

Palmeri M, Funel N, Di Franco G, Furbetta N, Gianardi D, Guadagni S, Bianchini M, Pollina LE, Ricci C, Del Chiaro M, Di Candio G, Morelli L

Retrospective Cohort Study

6837 Feasibility and nutritional impact of laparoscopic assisted tailored subtotal gastrectomy for middle-third gastric cancer

Liu H, Jin P, Ma FH, Ma S, Xie YB, Li Y, Li WK, Kang WZ, Tian YT

6853 Role of doublecortin-like kinase 1 and leucine-rich repeat-containing G-protein-coupled receptor 5 in patients with stage II/III colorectal cancer: Cancer progression and prognosis

Kang XL, He LR, Chen YL, Wang SB



World Journal of Gastroenterology

Contents

Weekly Volume 26 Number 43 November 21, 2020

Clinical Trials Study

6867 Comparison of two supplemental oxygen methods during gastroscopy with propofol mono-sedation in patients with a normal body mass index

Shao LJZ, Zou Y, Liu FK, Wan L, Liu SH, Hong FX, Xue FS

Observational Study

Barriers for resuming endoscopy service in the context of COVID-19 pandemic: A multicenter survey from 6880 Egypt

Elshaarawy O, Lashen SA, Makhlouf NA, Abdeltawab D, Zaghloul MS, Ahmed RM, Fathy H, Afifi S, Abdel-Gawad M, Abdelsameea E, Abd-Elsalam S, Mohamed SY, Tag-Adeen M, Tharwat M, Alzamzamy A, Bekhit AN, Eid AM, Awad A, Aamr M, Abd El Dayem WA, Wifi MN, Alboraie M

SYSTEMATIC REVIEWS

6891 Crohn's disease in low and lower-middle income countries: A scoping review

Rajbhandari R, Blakemore S, Gupta N, Adler AJ, Noble CA, Mannan S, Nikolli K, Yih A, Joshi S, Bukhman G



Contents

Weekly Volume 26 Number 43 November 21, 2020

ABOUT COVER

Editorial Board Member of World Journal of Gastroenterology, Dr. Vasiliy I Reshetnyak is a Distinguished Professor at the AI Yevdokimov Moscow State University of Medicine and Dentistry in Moscow, Russia. In 1977, he graduated with honors from the Medical Faculty of IM Sechenov 1st Moscow Medical Institute. In 1982m Dr. Reshetnyak defended his PhD thesis. Working at the Central Research Institute of Gastroenterology (CRIG) led to obtainment of his doctoral thesis (DSc) in 1996 and his rise to Deputy Director on Scientific Work at the CRIG. Since 2003, Professor Reshetnyak has served as an Academic Secretary of the Research Institute of General Reanimatology. His scientific research activity has focused on the diagnosis, clinical and laboratory manifestations, and treatment of gastroenterological diseases with patients in critical states. Since 2019, Dr. Reshetnyak has held the rank of Professor of the Department of Propaedeutic of Internal Diseases and Gastroenterology. (L-Editor: Filipodia)

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ORIGINAL ARTICLE

Retrospective Cohort Study

Role of doublecortin-like kinase 1 and leucine-rich repeat-containing G-protein-coupled receptor 5 in patients with stage II/III colorectal cancer: Cancer progression and prognosis

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Author contributions: Kang XL was responsible for the statistical analysis and preparation of manuscript; He LR provided information of the surgical patients; Chen YL provided the patient's pathological and immunohistochemical staining sections; Wang SB is the guarantor of this work and as such, has full access to all the data in the study and takes responsibility for the integrity of the data.

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Abstract

BACKGROUND

Cancer stem cells (CSCs) are a subpopulation of cancer cells with the potential of self-renewal and differentiation. CSCs play critical roles in tumorigenesis, recurrence, metastasis, radiation tolerance and chemoresistance.

AIM

To assess the expression patterns and clinical potential of doublecortin-like kinase 1 (DCLK1) and leucine-rich repeat-containing G-protein-coupled receptor 5 (Lgr5), as prognostic CSC markers of colorectal cancer (CRC).

METHODS

The expression of DCLK1 and Lgr5 in CRC tissue sections from 92 patients was determined by immunohistochemistry. Each case was evaluated using a combined scoring method based on signal intensity staining (scored 0-3) and the



Board.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

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proportion of positively stained cancer cells (scored 0-3). The final staining score was calculated as the intensity score multiplied by the proportion score. Low expression of DCLK1 and Lgr5 was defined as a score of 0-3; high expression of DCLK1 and Lgr5 was defined as a score of \geq 4. Specimens were categorized as either high or low expression, and the correlation between the expression of DCLK1 or Lgr5 and clinicopathological factors was investigated.

RESULTS

DCLK1 and Lgr5 expression levels were significantly positively correlated. CRC patients with high DCLK1, Lgr5 and DCLK1/Lgr5 expressions had poorer progression-free survival and overall survival. Moreover, high expression of DCLK1 was an independent prognostic factor for recurrence and overall survival in patients with CRC by multivariate analysis (P = 0.026 and P = 0.049, respectively).

CONCLUSION

DCLK1 may be a potential CSC marker for the recurrence and survival of CRC patients.

Key Words: Colorectal cancer; Cancer stem cells; Doublecortin-like kinase 1; Leucine-rich repeat-containing G-protein-coupled receptor 5; Cancer prognosis; Cancer progression

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Core Tip: The role of doublecortin-like kinase 1 (DCLK1) and leucine-rich repeatcontaining G-protein-coupled receptor 5 (Lgr5) in patients with stage II/III colorectal cancer (CRC) remains uncertain. In this study, we found a positive correlation between the expression of DCLK1 and Lgr5, suggesting that DCLK1 and Lgr5 were involved in the malignant pathological development of CRC. High DCLK1 expression could predict the risk of recurrence and survival in CRC patients after surgery, which may be used as a potential cancer stem cells marker for the recurrence and survival of stage II/III CRC patients.

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INTRODUCTION

Colorectal cancer (CRC) is the third most common malignant tumor worldwide, with 1.8 million new cases annually. In China, CRC has caused more than 800000 deaths, and its incidence is increasing every year^[1,2]. Despite significant improvements in the management of CRC, distant metastases and relapse remain the major causes of patient mortality. Cancer stem cells (CSCs) are a subpopulation of cancer cells with the potential of self-renewal and differentiation. CSCs play critical roles in tumorigenesis, recurrence, metastasis, radiation tolerance and chemoresistance.

Doublecortin-like kinase 1 (DCLK1), a microtubule-associated protein, has been regarded as a CSC marker receiving considerable attention. Nakanishi et al^[3] showed that DCLK1-positive CRC cells mark a subset of tumor cells with higher potential for tumor initiation, sphere formation and *in vivo* tumorigenicity. DCLK1 distinguishes CSCs from normal stem cells in CRC. Combination treatment with fluorouracil (5-FU) and the DCLK1 inhibitor, LRRK2-IN-1 (LRRK), decreased 5-FU-induced phosphorylation of Chk1 and canceled 5-FU-induced cell-cycle arrest at the S phase. Suehiro et al^[4] suggested that a combination of 5-FU and LRRK may be an effective, novel approach for the treatment of CRC. DCLK1-positive tumor cells exhibited spheroid formation and tumorigenesis in mouse pancreas^[5]. Targeting DCLK1expressing cells in hepatocellular and pancreatic carcinoma revealed that this marker



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may be a reliable molecule in targeted therapeutic strategies^[6,7]. However, contradictory observations have been reported in which patients with CRC exhibiting high DCLK1 expression had longer survival times than patients with low DCLK1 expression^[8]. Further study may be needed to define the role of DCLK1 in CRC development and progression.

Leucine-rich repeat-containing G-protein-coupled receptor 5 (Lgr5) belongs to the family of G-protein-coupled receptors, which contain 17 Leucine-rich repeats and a transmembrane domain containing an α -helix. Lgr5 is a marker of normal intestinal stem cells and colorectal CSCs^[9,10]. Lgr5 plays an important role in the pathogenesis of gastric cancer and CRC^[11-13], and Lgr5 expression is closely related to tumorigenesis, chemotherapy resistance and recurrence of gastric cancer and CRC^[14,15]. High Lgr5 expression is associated with a poor prognosis in stage IV CRC^[16]. Therefore, Lgr5 is considered an indicator of poor outcome and is a potential target of CRC; however, other reports have shown increased Lgr5 expression in well-differentiated and earlystage gastric carcinomas^[17,18].

Designing novel targeting drugs based on specific CSC markers is the goal of CSC therapy. Due to the lack of clear understanding of the potency of DCLK1 in CRC in previous studies, its expression and clinical significance were determined in an extensive collection of CRC samples. Additionally, we evaluated the potential CSC marker, Lgr5, in the same series of CRC samples, considering the possible similarities between gastric cancer and CRC. The aim of this study was to identify the relationship between DCLK1 and Lgr5 in CRC, determine their clinical significance as CSC markers for the recurrence and survival of stage II/III CRC patients, and lay the foundation for further study on the role of DCLK1 and Lgr5 in CRC stem cells. To our knowledge, this is the first study to investigate the relationship between clinicopathological parameters and the prognostic value of these CSC markers in CRC.

MATERIALS AND METHODS

Clinical data

In total, 92 patients with CRC from Peking University, Shenzhen Hospital, from August 2007 to February 2016 were studied. All patients were pathologically confirmed to have stage II/III CRC and had surgical tumor or nontumor tissues stored before therapy. Clinicopathological parameters, including age, gender, and depth of penetration, lymph node metastasis, pathological tumor-node-metastasis (TNM) stage, tumor differentiation, and primary tumor site were documented in a database and were fully anonymous throughout the study (Table 1). We excluded patients if they had previous chemoradiotherapy treatment or metachronous or synchronous cancers. Patients' clinical data were retrospectively obtained from their medical records, and the last follow-up was in March 2019.

Immunohistochemistry

A total of 92 formalin-fixed, paraffin-embedded samples were cut into sections 5-µm thick and stained using a standard-chain polymer-conjugated technique. The tissue sections were dewaxed, antigens were retrieved in an autoclave for 10 min, and the sections were then cooled to room temperature. Endogenous peroxidases were blocked by incubating the sections in 3% hydrogen peroxide (kit-0014, Maxim, Fuzhou, China). The sections were then incubated overnight at 4 °C with the following primary antibodies: Rabbit polyclonal anti-DCLK1 (1:100 dilution, ab31704; Abcam, United Kingdom) and rabbit polyclonal anti-Lgr5 (1:50 dilution, ab75732; Abcam, United Kingdom). The slides were stained and visualized with a standard immunohistochemistry kit (kit-0014, Maxim, Fuzhou, China). Colorectal cancer tissues with intense immunoreactivity to DCLK1 and Lgr5 were used as positive controls; in the negative control, the primary antibody was replaced with phosphate-buffered saline (PBS).

Scoring system

DCLK1 and Lgr5 staining was evaluated using a coded semiquantitative scoring system, and the evaluator was blinded to the clinical and pathological parameters^[19]. A pathologist diagnosed the samples, and two observers scored the immunostained slides semiquantitatively after examining a series on a double-headed microscope. A pathologist also confirmed the results to provide a comprehensive review of section staining. Initially, the slides were scanned at 10 × magnification to obtain a general impression of the overall tumor cell distribution^[20]. Positive cells were then assessed



Table 1 Correlation of immunoreactivity of doublecortin-like kinase 1 and leucine-rich repeat-containing G-protein-coupled receptor 5 with clinicopathological features

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MSI 3 (3) 2 (67) 1 (33) 2 (67) 1 (33) Recurrence	MSI							
Recurrence	MSS	89 (97)	46 (52)	43 (48)	0.609	60 (67)	29 (33)	0.978
	MSI	3 (3)	2 (67)	1 (33)		2 (67)	1 (33)	
Absent 67 (73) 37 (55) 30 (45) 0.338 44 (66) 23 (34) 0.565	Recurrence							
	Absent	67 (73)	37 (55)	30 (45)	0.338	44 (66)	23 (34)	0.565



Present	25 (27)	11 (44)	14 (56)		18 (72)	7 (28)	
Lung metastasis							
Absent	80 (87)	40 (50)	40 (50)	0.281	52 (65)	28 (35)	0.206
Present	12 (13)	8 (67)	4 (33)		10 (83)	2 (17)	
Liver metastasis							
Absent	83 (90)	42 (51)	41 (49)	0.359	55 (66)	28 (34)	0.484
Present	9 (10)	6 (67)	3 (33)		7 (78)	2 (22)	

DCLK1: Doublecortin-like kinase 1; Lgr5: Leucine-rich repeat-containing G-protein-coupled receptor 5; TNM: Tumor-node-metastasis.

semiquantitatively at higher magnifications, and the final scores were determined. DCLK1 and Lgr5 expression levels in CRC were assessed using three scoring methods: Staining intensity, proportion of positive cells and final score. The immunostaining intensity was divided into four categories: 0 (no immunostaining), 1 (weak immunostaining), 2 (moderate immunostaining) and 3 (strong immunostaining). Using the proportion of positive cells, the protein expression levels were semiquantitated and scored from 0 to 3 as follows: 0 (positive cells < 5%), 1 (positive cells 5%-30%), 2 (positive cells 31%-60%), and 3 (positive cells > 60%). The final staining score was calculated as the intensity score multiplied by the proportion score, and a score of 0, 1, 2, 3, 4, 6 and 9 was given^[21]. Low expression of DCLK1 and Lgr5 was defined as a score of 0-3; high expression of DCLK1 and Lgr5 was defined as a score of ≥ 4 .

Statistical analysis

The software SPSS (version 16, United States) was used to analyze the findings. Pearson's Chi-square and Spearman's correlation tests were applied to evaluate the correlation between DCLK1 and Lgr5 expression and clinicopathological parameters. Cumulative survival of the patients was estimated using the Kaplan-Meier method, and the significance of the survival differences was tested using the log rank test. Multivariate analysis was performed using a Cox proportional hazards regression model to examine the interaction between DCLK1 and Lgr5 expression and other clinicopathological variables and to estimate the independent prognostic effect of DCLK1 and Lgr5 on survival by adjusting for confounding factors. A difference of P <0.05 between the groups was considered statistically significant.

RESULTS

Patient and clinicopathological characteristics

The mean age of the 92 CRC patients was 49 years (range 15-79 years). Fifty-two patients were male (57%). Eighteen CRC patients (20%) showed T3 depth of penetration, whereas 74 patients (80%) showed T4. For the patients with available regional lymph node metastasis, 38 (41%) were category N0, 41 (45%) were N1, and 13 (14%) were N2. Based on tumor distant metastasis staging, 38 CRC cases (41%) were stage II and 54 (59%) were stage III. In terms of tumor differentiation, 66 (72%) and 26 (28%) cases had well/moderate and poor/mucinous differentiation, respectively. Forty-two tumors (46%) were in the left colon, 17 (18%) were in the right colon, and 33 (36%) were in the rectum. Table 1 summarizes the clinicopathological characteristics of these patients. The relationships between preoperative CEA/CA19-9/adjuvant chemotherapy/MSI/recurrence/lung metastases/liver metastases and DCLK1/Lgr5 are shown in Tables 1 and 2.

DCLK1 and Lgr5 expression and association with clinicopathological parameters

Immunohistochemical findings showed that DCLK1 expression was mainly localized in the membranous area of CRC cells. Low DCLK1 expression was found in 48 cases (52%), while high DCLK1 expression was seen in 44 cases (48%) (Figure 1). DCLK1 expression and clinicopathological parameters were not significantly correlated (Table 1). Immunodetection of Lgr5 expression showed that it was generally localized in the cytoplasmic area of tumor cells. In the light of the final score, 67% of cases (62/92) displayed low Lgr5 expression (score of 0-3), and 33% showed high Lgr5



Table 2 Correlation of immunoreactivity of doublecortin-like kinase 1/leucine-rich repeat-containing G-protein-coupled receptor 5 with clinicopathological features

Clinicopathologic		DCLK1/Lgr5 phenotypes				
characteristics	n (%)	DCLK1 ^{Low} /Lgr5 ^{Low}	DCLK1 ^{Low} /Lgr5 ^{High}	DCLK1 ^{High} /Lgr5 ^{Low}	DCLK1 ^{High} /Lgr5 ^{High}	P value
Age (yr)						
≤ 49	49 (53)	23 (47)	3 (6)	10 (20)	13 (27)	0.890
> 49	43 (47)	18 (42)	4 (9)	11 (26)	10 (23)	
Gender						
Male	52 (57)	25 (48)	4 (8)	12 (23)	11 (21)	0.348
Female	40 (43)	16 (40)	3 (7)	9 (23)	12 (30)	
Depth of penetration						
T3	18 (20)	5 (28)	1 (6)	8 (44)	4 (22)	0.223
T4	74 (80)	36 (49)	6 (8)	13 (17)	19 (26)	
Lymph node						
N0	38 (41)	17 (45)	3 (8)	10 (26)	8 (21)	0.858
N1	41 (45)	18 (44)	4 (10)	9 (22)	10 (24)	
N2	13 (14)	6 (46)	0 (0)	2 (15)	5 (39)	
Tumor stage (TNM)						
Π	38 (41)	17 (45)	3 (8)	10 (26)	8 (21)	0.774
III	54 (59)	24 (45)	4 (7)	11 (20)	15 (28)	
Differentiation						
Well/moderate	66 (72)	32 (48)	5 (8)	16 (24)	13 (20)	0.115
Poor/mucinous	26 (28)	9 (35)	2 (8)	5 (19)	10 (38)	
Tumour site						
Left	42 (46)	22 (52)	2 (5)	10 (24)	8 (19)	0.187
Right	17 (18)	4 (23)	3 (18)	3 (18)	7 (41)	
Rectum	33 (36)	15 (46)	2 (6)	8 (24)	8 (24)	
Postoperative adjuvant chemotherapy						
Xeloda monotherapy	7 (7)	3 (43)	1 (14)	21 (29)	22 (14)	0.875
FOLFOX regimen	32 (35)	15 (47)	3 (9)	5 (16)	9 (28)	
XELOX regimen	53 (58)	23 (43)	3 (6)	14 (26)	13 (25)	
CEA						
Normal	68 (74)	33 (49)	5 (7)	12 (18)	18 (26)	0.238
High	24 (26)	8 (33)	2 (8)	9 (38)	5 (21)	
CA19-9						
Normal	66 (72)	29 (44)	6 (9)	16 (24)	15 (23)	0.710
High	26 (28)	12 (46)	1 (4)	5 (19)	8 (31)	
MSI						
MSS	89 (97)	39 (44)	7 (8)	21 (25)	22 (23)	0.713
MSI	3 (3)	2 (67)	0 (0)	0 (0)	1 (33)	
Recurrence						
Absent	67 (73)	30 (45)	7 (10)	14 (21)	16 (24)	0.374

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Present	25 (27)	11 (44)	0 (0)	7 (28)	7 (28)	
Lung metastasis						
Absent	80 (87)	33 (41)	7 (9)	19 (24)	21 (26)	0.365
Present	12 (13)	8 (66)	0 (0)	2 (17)	2 (17)	
Liver metastasis						
Absent	83 (90)	35 (42)	7 (9)	20 (24)	2 (25)	0.478
Present	9 (10)	6 (67)	0 (0)	1 (11)	2 (22)	

DCLK1: Doublecortin-like kinase 1; Lgr5: Leucine-rich repeat-containing G-protein-coupled receptor 5; TNM: Tumor-node-metastasis.

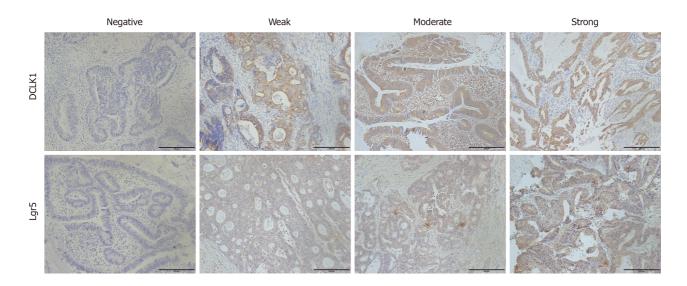


Figure 1 Representative doublecortin-like kinase 1 and leucine-rich repeat-containing G-protein-coupled receptor 5 immunohistochemical staining in colorectal cancer samples. Doublecortin-like kinase 1 (DCLK1) was expressed at the membrane, and leucine-rich repeatcontaining G-protein-coupled receptor 5 (Lgr5) was expressed in the cytoplasm of colorectal cancer cells. Expression levels of DCLK1 and Lgr5 were scored as negative (0), weak (1), moderate (2), or strong (3) staining intensity (original magnification 200 ×). DCLK1: Doublecortin-like kinase 1; Lgr5: Leucine-rich repeatcontaining G-protein-coupled receptor 5.

> expression (Figure 1). Our analysis indicated that patients with primary tumor in the left colon had higher Lgr5 expression levels (P = 0.032) (Table 1). Lgr5 expression and other clinicopathological parameters were not significantly correlated (Table 1).

Combined analysis of DCLK1 and Lgr5 expression in CRC

A comparison of the expression patterns of DCLK1 and Lgr5 markers showed that these two markers had reciprocal significant correlation in the same series of CRC samples (P < 0.001, Figure 2). Among the 92 CRC samples, 41 (44%) showed the DCLK1^{Low}/Lgr5^{Low} phenotype, 7 (8%) showed the DCLK1^{Low}/Lgr5^{High} phenotype, 21 (23%) showed the DCLK1^{High}/Lgr5^{Low} phenotype, and 23 (25%) showed the DCLK1^{High} /Lgr5^{High} phenotype (Table 2). One-way analysis of variance (ANOVA) and Tukey's post hoc analysis were used to calculate the correlation between DCLK1/Lgr5 phenotypic expression and clinicopathological parameters. It was found that the DCLK1/Lgr5 phenotypic expression was not significantly associated with clinicopathological variables (Table 2).

Progression-free and overall survival analysis in CRC patients

Progression-free survival (PFS) and overall survival (OS) differed significantly according to the immunoreactivity of DCLK1 and Lgr5 expression levels. Analysis using the Kaplan-Meier method and log-rank tests showed a lower survival rate in the DCLK1^{High} group compared with the DCLK1^{Low} group (PFS: P = 0.003, OS: P = 0.003, Figure 3A and B). The prognostic impact of Lgr5 expression in CRC was similar to that of DCLK1 (PFS: *P* = 0.037, OS: *P* = 0.036, Figure 3C and D). Moreover, patients with coexpression of DCLK1^{High}/Lgr5^{High} had a poorer prognosis than the other groups (PFS: P= 0.003, OS: P = 0.008, Figure 3E and F), including the DCLK1^{Low}/Lgr5^{Low}, DCLK1^{Low}



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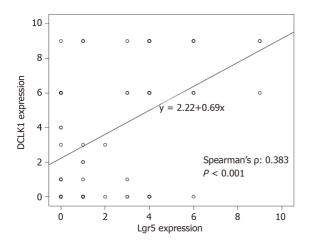


Figure 2 Correlation between doublecortin-like kinase 1 and leucine-rich repeat-containing G-protein-coupled receptor 5 immunohistochemical expression in colorectal cancer specimens. Doublecortin-like kinase 1 (DCLK1) and leucine-rich repeat-containing G-protein-coupled receptor 5 expression were positively significantly correlated (r = 0.206, P < 0.001). DCLK1: Doublecortin-like kinase 1; Lgr5: Leucine-rich repeat-containing Gprotein-coupled receptor 5.

/Lgr5^{High}, and DCLK1^{High}/Lgr5^{Low} phenotypes.

Predictive value of DCLK1 and Lgr5 expression levels for recurrence and survival

A Cox proportional hazards model was used to estimate the effect of DCLK1 and Lgr5 expression on recurrence and survival. Univariate analysis showed that the following factors were significantly related to postoperative recurrence and OS: Tumor stage, differentiation, CEA, CA19-9, DCLK1, Lgr5 and DCLK1/Lgr5 expression level (P =0.022, 0.037, 0.001, 0.002, 0.006, 0.044, and 0.006 for recurrence, *P* = 0.017, 0.031, 0.002, 0.005, 0.006, 0.043, and 0.012 for OS, respectively). Multivariate analysis confirmed that DCLK1 expression level was an independent predictor of recurrence and OS in patients with CRC (hazard ratio = 4.656, 95% confidence interval = 1.207-17.956, P = 0.026 for recurrence, hazard ratio = 4.272, 95% confidence interval = 1.005-18.167, P = 0.049 for OS; Tables 3 and 4).

DISCUSSION

Few studies have reported the correlation between DCLK1 and Lgr5 in CRC. The results of this study showed that DCLK1 and Lgr5 immunoreactivity was observed in 53% and 41% of CRC cases, respectively, using standard immunostaining. DCLK1 and Lgr5 expression were positively correlated in CRC tissues. Therefore, DCLK1 may affect Lgr5 expression via an unknown mechanism^[3,22,23], and the two complement each other to participate in CRC development, invasion and metastasis^[3,24,25]. It was found that patients with DCLK1^{High}, Lgr5^{High} and DCLK1^{High}/Lgr5^{High} expression had poorer PFS and OS, which implied that high DCLK1 and Lgr5 expression may specifically predict the most aggressive and fatal types of primary CRC in cases with stage II/III disease^[12,26-29]. DCLK1 and Lgr5 are targets of Wnt signaling, which has emerged as a critical regulator of stem cells, and its pathway is integral in both stem cell and cancer cell maintenance and growth^[30,31]. Gastric cancer patients with high DCLK1 expression were shown to have significantly shorter PFS and OS^[26]. Uchida confirmed that Lgr5 was observed in both early and late events in colorectal tumorigenesis^[12]. DCLK1 may be associated with Lgr5 during CRC development. Conversely, contradictory evidence has shown that DCLK1 overexpression is significantly associated with better PFS and OS^[32], and increased Lgr5 expression in well-differentiated and early-stage gastric carcinomas^[17,18]. The exact regulatory mechanism between DCLK1 and Lgr5 requires further exploration.

Despite no association between DCLK1 expression and TNM stage or degree of tissue differentiation being found, DCLK1 was shown to be a potential predictor of tumor recurrence and survival in patients with stage II/III CRC by univariate and multivariate Cox regression analyses, reflecting a more invasive tumor phenotype^[23,33]. The discovery of CSCs suggests intratumoral heterogeneity^[34]. High DCLK1 expression levels indicate more invasive CRC, resulting in recurrence and metastasis, which may be related to the CSC characteristics of some cancer cells^[35]. Other



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Table 3 Prognostic value, univariate and multivariate analyses of potential predictor for recurrence					
Variables	HR	95%CI	P value		
Univariate					
Age (≤ 49 <i>vs</i> > 49 yr)	0.742	0.303-1.818	0.515		
Gender (Male vs Female)	0.758	0.302-1.901	0.555		
Depth of penetration (T3 vs pT4)	1.310	0.383-4.476	0.006		
Lymph node					
N0	1 (ref)				
N1	2.590	0.795-8.439	0.114		
N2	7.562	2.189-26.115	0.001		
Tumor stage (TNM) (II vs III)	3.610	1.201-10.849	0.022		
Differentiation (well/moderate vs poor/mucinous)	2.561	1.058-6.198	0.037		
Tumor site					
Left	1 (ref)				
Right	0.735	0.205-2.636	0.637		
Rectum	0.834	0.307-2.263	0.721		
CEA (normal vs high)	4.722	1.933-11.535	0.001		
CA19-9 (normal vs high)	3.961	1.633-9.606	0.002		
MSI (MSS vs MSI)	0.047	0.000-1327.112	0.559		
Lung metastasis (absent vs present)	2.325	0.844-6.405	0.103		
Liver metastasis (absent vs present)	2.351	0.785-7.038	0.127		
DCLK1 (low vs high)	3.958	1.496-10.472	0.006		
Lgr5 (low vs high)	2.521	1.025-6.199	0.044		
DCLK1/Lgr5 (others ¹ vs high/high)	3.808	1.471-9.854	0.006		
Multivariate					
Tumor stage (TNM) (II vs III)	6.846	1.918-24.445	0.003		
Differentiation (well/moderate vs poor/mucinous)	1.864	0.699-4.971	0.241		
CEA (normal vs high)	4.835	1.651-14.154	0.004		
CA19-9 (normal vs high)	4.102	1.529-11.005	0.005		
DCLK1 (low vs high)	4.656	1.207-17.956	0.026		
Lgr5 (low vs high)	0.764	0.076-7.691	0.819		
DCLK1/Lgr5 (others ¹ vs high/high)	3.429	0.212-55.583	0.386		

¹Low/low low/high and high/low. HR: Hazard ratio; CI: Confidence interval; DCLK1: Doublecortin-like kinase 1; Lgr5: Leucine-rich repeat-containing Gprotein-coupled receptor 5; TNM: Tumor-node-metastasis.

candidate CSC markers, such as Lgr5, ALDH1, and Musashi-1, also suggest a correlation between tumor expression and poor prognosis in CRC^[36-38]. The present study results are based only on immunohistochemical analysis, which does not quantify RNA or protein expression. Therefore, future studies should quantitatively evaluate DCLK1 using reverse transcriptase polymerase chain reaction or fluorescence-activated cell sorting in both normal and CRC tissues.

Although several putative CRC stem cell markers have been identified, how these markers can be used clinically remains unclear. One study applied nanoparticle-based DCLK1 small interfering RNA in colorectal tumor xenografts, which inhibited tumor growth and downregulated c-Myc and Notch1 expression^[24]. Sureban found that small interfering RNA blockage of DCLK1 reduced *in vivo* tumorigenic potential in CRC. This function was mediated by reducing the primary transcript of MIRLET7 and

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Table 4 Prognostic value, univariate and multivariate analyses of potential predictor for survival						
Variables	HR	95%CI	P value			
Univariate						
Age (≤ 49 <i>vs</i> > 49 yr)	0.681	0.276-1.684	0.406			
Gender (Male vs Female)	0.795	0.316-2.001	0.626			
Depth of penetration (T3 vs pT4)	0.772	0.226-2.640	0.680			
.ymph node						
10	1 (ref)					
J1	2.774	0.839-9.170	0.094			
J2	8.899	2.496-31.723	0.001			
umor stage (TNM) (II vs III)	3.880	1.274-11.871	0.017			
Differentiation (well/moderate vs poor/mucinous)	0.376	0.155-0.913	0.031			
umour site						
eft	1 (ref)					
light	0.798	0.221-2.880	0.731			
lectum	0.872	0.319-2.384	0.790			
EA (normal <i>vs</i> high)	0.245	0.101-0.595	0.002			
A19-9 (normal vs high)	3.569	1.477-8.621	0.005			
ISI (MSS vs MSI)	0.047	0.000-3055.106	0.588			
ecurrence (absent vs present)	8.038	3.070-21.050	0.001			
ung metastasis (absent <i>vs</i> present)	2.141	0.776-5.911	0.142			
iver metastasis (absent <i>vs</i> present)	2.216	0.734-6.689	0.158			
OCLK1 (low <i>vs</i> high)	4.167	1.491-11.644	0.006			
gr5 (low <i>vs</i> high)	2.575	1.030-6.438	0.043			
DCLK1/Lgr5 (others ¹ vs high/high)	3.273	1.297-8.262	0.012			
ſultivariate						
umor stage (TNM) (II vs III)	6.087	1.647-22.499	0.007			
ifferentiation (well/moderate vs poor/mucinous)	2.706	0.989-7.406	0.053			
ΈΑ (normal <i>vs</i> high)	4.363	1.346-14.136	0.014			
A19-9 (normal vs high)	1.078	0.362-3.208	0.893			
ecurrence (absent vs present)	12.002	3.066-46.988	0.001			
CLK1 (low <i>vs</i> high)	4.272	1.005-18.167	0.049			
gr5 (low <i>vs</i> high)	7.088	0.582-86.342	0.125			
DCLK1/Lgr5 (others ¹ vs high/high)	0.593	0.039-9.085	0.708			

¹Low/low low/high and high/low. HR: Hazard ratio; CI: Confidence interval; DCLK1: Doublecortin-like kinase 1; Lgr5: Leucine-rich repeat-containing Gprotein-coupled receptor 5; TNM: Tumor-node-metastasis.

increasing c-Myc expression, both of which are related to the loss of epithelial differentiation^[39]. CSCs are widely chemoresistant and radioresistant, which is a key factor in treatment resistance and cancer recurrence^[40-42]. These studies suggest that DCLK1 may be a CSC marker with a functional role and thus may be an important therapeutic target.

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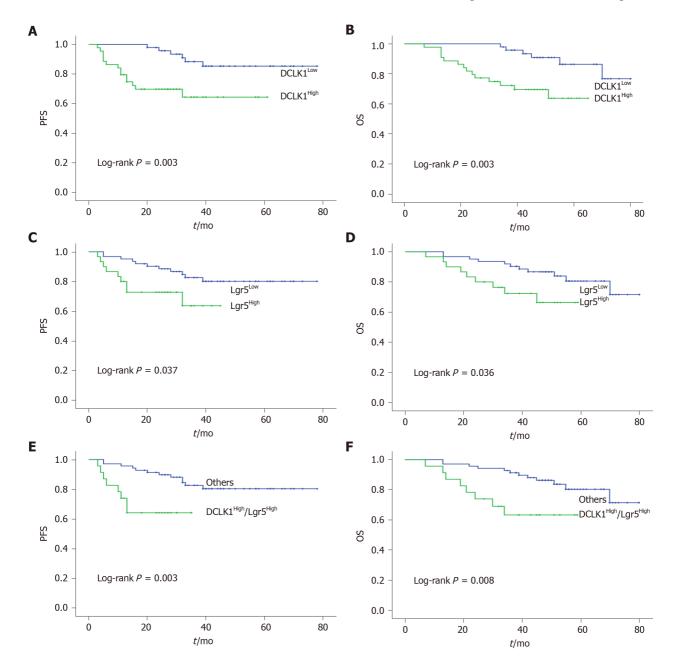


Figure 3 High expression of doublecortin-like kinase 1 and leucine-rich repeat-containing G-protein-coupled receptor 5 was significantly associated with poorer progression-free survival and overall survival in patients with colorectal cancer. Cumulative progression-free survival and overall survival of colorectal cancer patients in different groups were compared. A and B: Doublecortin-like kinase 1 (DCLK1)^{High} and DCLK1^{Low}; C and D: Leucine-rich repeat-containing G-protein-coupled receptor 5 (Lgr5)^{High} and Lgr5^{Low}; E and F: DCLK1^{High}/Lgr5^{High} and others. DCLK1: Doublecortin-like kinase 1; Lgr5: Leucine-rich repeat-containing G-protein-coupled receptor 5; PFS: Progression-free survival; OS: Overall survival.

CONCLUSION

In summary, this study found a positive correlation between the expression of DCLK1 and Lgr5, suggesting that DCLK1 and Lgr5 are involved in the malignant pathological development of CRC. DCLK1^{High}, Lgr5^{High} and DCLK1^{High}/Lgr5^{High} expression resulted in poorer PFS and OS in patients with stage II/III CRC. High DCLK1 expression could predict the risk of recurrence and survival in CRC patients after surgery, which may be used as a potential CSC marker for the recurrence and survival of stage II/III CRC patients. However, further analysis is required to investigate the CSC marker, DCLK1, as a potential early diagnostic and therapeutic target for CRC.

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ARTICLE HIGHLIGHTS

Research background

The role of doublecortin-like kinase 1 (DCLK1) and leucine-rich repeat-containing Gprotein-coupled receptor 5 (Lgr5) in patients with stage II/III colorectal cancer (CRC) remains uncertain.

Research motivation

Designing novel targeting drugs based on specific cancer stem cell (CSC) markers is the goal of CSC therapy. Due to the lack of clear understanding of the potency of DCLK1 in CRC in previous studies, its expression and clinical significance were determined in an extensive collection of CRC samples.

Research objectives

We determined the clinical significance of DCLK1 and Lgr5 as CSC markers for the recurrence and survival of stage II/III CRC patients.

Research methods

The expression of DCLK1 and Lgr5 in CRC tissue sections from 92 patients was detected by immunohistochemistry.

Research results

In this study, we have found a positive correlation between the expression of DCLK1 and Lgr5, suggesting that DCLK1 and Lgr5 are involved in the malignant pathological development of CRC. High DCLK1 expression could predict the risk of recurrence and survival in CRC patients after surgery.

Research conclusions

DCLK1 may be a potential CSC marker for the recurrence and survival of CRC patients.

Research perspectives

Our research raises the possibility that treatments targeting DCLK1 could be beneficial in CRC patients.

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