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ORIGINAL ARTICLE

Retrospective Study

High patatin like phospholipase domain containing 8 expression as a biomarker for poor prognosis of colorectal cancer

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Abstract

BACKGROUND

Patatin like phospholipase domain containing 8 (PNPLA8) has been shown to play a significant role in various cancer entities. Previous studies have focused on its roles as an antioxidant and in lipid peroxidation. However, the role of PNPLA8 in colorectal cancer (CRC) progression is unclear.

AIM

To explore the prognostic effects of PNPLA8 expression in CRC.

METHODS

A retrospective cohort containing 751 consecutive CRC patients was enrolled. PNPLA8 expression in tumor samples was evaluated by immunohistochemistry staining and semi-quantitated with immunoreactive scores. CRC patients were divided into high and low PNPLA8 expression groups based on the cut-off values, which were calculated by X-tile software. The prognostic value of PNPLA8 was identified using univariate and multivariate Cox regression analysis. The over-all survival (OS) rates of CRC patients in the study cohort were compared with Kaplan-Meier analysis and Log-rank test.

RESULTS

PNPLA8 expression was significantly associated with distant metastases in our cohort (P = 0.048). CRC patients with high PNPLA8 expression indicated poor OS (median OS = 35.3, P = 0.005). CRC patients with a higher PNPLA8 expression at either stage I and II or stage III and IV had statistically significant shorter OS. For patients with left-sided colon and rectal cancer, the survival curves of two PNPLA8-expression groups showed statistically significant differences. Multivariate analysis also confirmed that high PNPLA8 expression was an independent prognostic factor for overall survival (hazard ratio HR = 1.328, 95%CI: 1.016-1.734, P = 0.038).

CONCLUSION
PNPLA8 is a novel independent prognostic factor for CRC. These findings suggest that PNPLA8 is a potential target in clinical CRC management.

Key Words: Biomarker; Colorectal cancer; Expression level; Overall survival; Patatin like phospholipase domain containing 8; Prognosis

Core Tip: Patatin like phospholipase domain containing 8 (PNPLA8) has been shown to be associated with a variety of cancers, but its role in the progression of colorectal cancer (CRC) is unclear. In this study, 751 consecutive CRC patients were retrospectively analyzed. The results of this study indicate that PNPLA8 is a new independent prognostic factor for CRC. High PNPLA8 expression in CRC leads to impaired survival. These findings suggest that PNPLA8 is a potential target for clinical CRC therapy, providing important insights to help personalize therapy for CRC patients.

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INTRODUCTION

Colorectal cancer (CRC) is among the deadliest tumors[1]. The only curative treatment for localized CRC is surgery, and patients with lymph node metastases are usually advised to undergo adjuvant chemotherapy[2]. The relatively low 5-year survival rate of about 56.9% is further affected by inadequate screening methods and increasing resistance to chemotherapy during the clinical course[3,4]. Currently, several reliable prognostic factors are widely used in clinical practice, such as molecular subtype, therapeutic response to previous adjuvant chemotherapy, time between adjuvant therapy and metastasis development (shorter is associated with poorer prognosis), comorbidities, and frailty[5,6]. Therefore, considering the heterogeneity of CRC, it is essential to develop new prognostic and therapeutic strategies. However, widely accepted new prognostic biomarkers are scarce.

Patatin like phospholipase domain-containing protein (PNPLA8), also termed Ca^{2+}-independent phospholipase A2γ (iPLA2γ), is localized to the mitochondrial matrix, where it may manifest its unique activity to cleave phospholipid side-chains from both *sn-1* and *sn-2* positions, consequently releasing either saturated or unsaturated fatty acids, including oxidized fatty acids[7]. As a calcium-independent and membrane-bound phospholipase, PNPLA8 catalyzes the esterolytic cleavage of fatty acids from glycerophospholipids to yield free fatty acids and lysophospholipids, hence regulating membrane physical properties and the release of lipid second messengers and growth factors[8,9]. It is essential for maintaining efficient bioenergetic mitochondrial function by regulating mitochondrial membrane lipid metabolism and composition[9]. Mutations in the *PNPLA8* gene have been linked to multiple diseases such as mitochondrial myopathy with lactic acidosis and mitochondrial myopathy[10]. Recently, it was found that the dysregulation of iPLA2γ can therefore be a critical factor in the development of many diseases[11,12], including metabolic diseases and multiple cancers, such as colitis and CRC[13]. However, the expression status of PNPLA8 in CRC and its relationship with clinicopathological features and prognosis are largely unknown.

In this study, to investigate the potential biomarker value of PNPLA8, 751 cases of tumor samples from a cohort of CRC patients were selected to analyze PNPLA8 protein expression by immunohistochemical staining. Additionally, concentrated analyses on the correlations between PNPLA8 expression and overall survival of CRC patients in this cohort were conducted to unveil the prognostic significance of PNPLA8 in CRC. Our results suggest that higher PNPLA8 expression is an independent predictor for poor prognosis in CRC patients, which could be potentially used to guide the clinical management of CRC patients.

MATERIALS AND METHODS

Patients and specimens

A total of 751 patients with CRC that were admitted to Zhongshan Hospital, Fudan University (Shanghai, China) between May 2008 and November 2012 were retrospectively enrolled in this study. The inclusion criteria were as follows: (1) Receiving primary radical resection; (2) pathologically confirmed colorectal adenocarcinoma; (3) no treatment before surgery; and (4) clinicopathological data available. CRC patients with radical resections of synchronous liver metastases were also included. CRC cancer stages were defined according to the International Union Against Cancer/American Joint Committee on Cancer TNM classification 8th edition. The diagnostic procedures were concluded before the current study

was conducted. During the analysis, the observers were fully blinded to patient data. The median follow-up time of the patient cohort was 46.1 mo (IQR = 32.9-59.5).

This study was approved by the Clinical Research Ethics Committee of Zhongshan Hospital, Fudan University. Informed consent was acquired from all patients of the primary cohort for the acquisition of clinical and pathological information and the use of surgical specimens.

Immunohistochemistry

Formalin-fixed paraffin-embedded surgical specimens were used for tissue microarray (TMA) construction and subsequent immunohistochemistry study as described previously[14]. Standard procedures were used to determine PNPLA8 expression levels in CRC tumor samples. After being dried overnight at 37˚C and deparaffinized in xylene, the TMA slide was rehydrated through graded alcohol and then immersed in 3% hydrogen peroxide to block endogenous peroxidase activity. After that, the sections were pretreated in a microwave oven (14 min in sodium citrate buffer, pH 6) and then incubated with 10% normal goat serum for 30 min. Primary antibody (rabbit anti-human PNPLA8 polyclonal antibody, ab223726, Abcam; diluted 1:150) was applied overnight in a moist chamber at 4˚C. After the primary antibody was washed off with PBS, the secondary goat anti-rabbit antibody (ab6721, Abcam; diluted 1:10000) was applied. Reaction products were visualized by incubation with 3,3'-diaminobenzidine and counterstained with hematoxylin. Negative controls were treated identically, but with the primary antibody omitted. In addition, the paracancerous tissues were used as controls.

Evaluation of immunohistochemical staining

Two independent pathologists who were blinded to the clinical data evaluated the immunostaining and the results were averaged. In case of significant discrepancies, a final score was established by reassessment on a double-headed microscope and a third person was asked to re-score the results and choose the value with the closest score. The scores for PNPLA8 intensity were set as follows: '+++' was 3; '++' was 2; '+' was 1; and '−' was 0. The area scores for PNPLA8 expression were set as follows: '1' (0%-25% positive cells among all tumor cells), '2'(25%-50% positive cells), '3' (51%-75% positive cells), and '4' (more than 75% positive cells). The final score for PNPLA8 expression was the intensity score multiplied by the area score, resulting in a final score ranging from 0 to 12. Boundaries were based on the results from X-Tile Software (Yale University, version 3.6.1). A final score of 0-8 was considered low PNPLA8 expression, while 9-12 was considered high PNPLA8 expression.

Statistical analysis

The statistical analysis was performed using SPSS 23.0 (IBM, Armonk, NY, United States). The association between clinicopathological features and PNPLA8 expression were accessed by Chi-square test or Fisher's exact test as appropriate. Kaplan-Meier analysis and Log-rank test were performed to evaluate the relationship between PNPLA8 expression and overall survival (OS). Univariate Cox regression analysis was performed to identify the independent prognostic factors among clinicopathological features and other information. Those factors with $P < 0.1$ in univariate Cox regression analyses were included in the multivariate Cox regression analysis. A two-sided $P < 0.05$ was considered statistically significant. To obtain the best prognostic efficacy, the cut-off values of PNPLA8 score were calculated using X-Tile Software (Yale University, version 3.6.1) based on the OS data[15].

RESULTS

Clinicopathologic characteristics of the enrolled patients with CRC

The clinicopathologic characteristics of the enrolled CRC patients are listed in Table 1. Approximately half of the patients (53.7%) were over 60-years-old, and their ages ranged from 19 years to 90 years with a median age of 62 (SD, 12.3) years. The male to female ratio was 60.3:39.7. The patients with CEA value over 5 ng/mL accounted for 47.3% of total patients, while those with CA199 value more than 37 U/mL accounted for 19.2%. The tumor location was categorized as right-sided colon in 209 cases (27.8%), left-sided colon in 200 cases (26.6%), and rectum in 342 cases (45.6%). There were 323 cases (43%) with tumor size over 4.0 cm, while the majority of all the cases were non-mucinous in terms of primary histological type. For primary tumor differentiation, 497 cases (66.2%) had well/moderate differentiation, while 254 cases (33.8%) were poor/anaplastic in tumor differentiation. TNM Staging results showed that a small portion of patients (197 cases, 26.2%) were at active metastasis stage, whereas only 96 cases (12.8%) and 56 cases (7.5%) showed vascular invasion and nerve invasion, respectively.

Correlations between PNPLA8 expression and clinicopathological parameters

We next examined PNPLA8 expression in tumor samples using immunohistochemistry staining and scored each sample according to the staining intensity (Figure 1) and staining area. Out of 751 stained CRC specimens, 689 (91.7%) showed positive PNPLA8 expression. These 751 samples were categorized into a PNPLA8-low expression group and PNPLA8-high expression group, and the correlations between PNPLA8 expression and the clinicopathological parameters were analyzed (Table 2). A positive correlation was observed between high cytoplasmic PNPLA8 staining and M stage ($P = 0.048$). However, there were no significant correlations between PNPLA8 staining and other parameters ($P > 0.05$), including age, sex, CEA, CA199, tumor location, tumor size, primary histological type, primary differentiation, T stage, N stage, vascular invasion, and nerve invasion.

Table 1 Clinicopathologic characteristics of the colorectal cancer patients in this study

Clinicopathologic parameters	*n*	Percentage (%)
All patients	751	100.0
Age in yr		
≤ 60	348	46.3
> 60	403	53.7
Sex		
Male	453	60.3
Female	298	39.7
CEA in ng/mL		
≤ 5	396	52.7
> 5	355	47.3
CA199 in U/mL		
≤ 37	607	80.8
> 37	144	19.2
Tumor location		
Right-sided colon	209	27.8
Left-sided colon	200	26.6
Rectum	342	45.6
Tumor size in cm		
≤ 4.0	428	57.0
> 4.0	323	43.0
Primary histological type		
Non-mucinous	640	85.2
Mucinous	111	14.8
Primary differentiation		
Well/moderate	497	66.2
Poor/anaplastic	254	33.8
T stage		
T1	24	3.2
T2	132	17.6
T3	354	47.1
T4	241	32.1
N stage		
N0	410	54.6
N1	231	30.8
N2	110	14.6
Vascular invasion		
No	655	87.2
Yes	96	12.8
Nerve invasion		
No	695	92.5
Yes	56	7.5

M stage		
M0	554	73.8
M1	197	26.2

CA199: Carbohydrate antigen 19-9; CEA: Carcinoembryonic antigen; M: Presence of metastasis; N: Extent of tumor spread to the lymph nodes; T: Extent of the tumor (the size of the tumor and any spread of tumor into nearby tissue).

Table 2 Correlations between patatin like phospholipase domain containing 8 expression and clinicopathological parameters of colorectal cancer patients in this study

Variables	PNPLA8 expression		*P* value
	Low (%)	High (%)	
All patients	331	420	
Age in yr			0.811
≤ 60	155 (46.8)	193 (46.0)	
> 60	176 (53.2)	227 (54.0)	
Sex			0.375
Male	199 (60.1)	239 (56.9)	
Female	132 (39.9)	181 (43.1)	
CEA in ng/mL			0.821
≤ 5	173 (52.3)	223 (53.1)	
> 5	158 (47.7)	197 (46.9)	
CA199 in U/mL			0.900
≤ 37	267 (80.7)	340 (81.0)	
> 37	64 (19.3)	80 (19.0)	
Tumor location			0.434
Right-sided colon	97 (29.3)	112 (26.7)	
Left-sided colon	92 (27.8)	108 (25.7)	
Rectum	142 (42.9)	200 (47.6)	
Tumor size in cm			0.589
≤ 4.0	185 (55.9)	243 (57.9)	
> 4.0	146 (44.1)	177 (42.1)	
Primary histological type			0.848
Non-mucinous	283 (85.5)	357 (85.0)	
Mucinous	48 (14.5)	63 (15.0)	
Primary differentiation			0.160
Well/moderate	210 (63.4)	287 (68.3)	
Poor/anaplastic	121 (36.6)	133 (31.7)	
T stage			0.618
T1/T2	66 (19.9)	90 (21.4)	
T3/T4	265 (80.1)	330 (78.6)	
N stage			0.684
N0	180 (54.4)	230 (54.8)	
N1	106 (32.0)	125 (29.8)	

N2	45 (13.6)	65 (15.4)	
Vascular invasion			0.710
No	287 (86.7)	368 (87.6)	
Yes	44 (13.3)	52 (12.4)	
Nerve invasion			0.638
No	308 (93.1)	387 (92.1)	
Yes	23 (6.9)	33 (7.9)	
M stage			0.048
M0	256 (77.3)	298 (71.0)	
M1	75 (22.7)	122 (29.0)	

CA199: Carbohydrate antigen 19-9; CEA: Carcinoembryonic antigen; PNPLA8: Patatin like phospholipase domain containing 8; M: Presence of metastasis; N: Extent of tumor spread to the lymph nodes; T: Extent of the tumor (the size of the tumor and any spread of tumor into nearby tissue).

High PNPLA8 expression is associated with poor overall survival of CRC patients

To further substantiate the importance of high PNPLA8 expression in CRC progression, we compared the OS of CRC patients in our study cohort with differential PNPLA8 expression levels. The median follow-up OS of the CRC patients was 46.1 mo (IQR = 36.9-60.9). We found that PNPLA8 expression was statistically significantly associated with a shorter OS (HR 1.445; 43.1 mo for PNPLA8-low group *vs* 35.4 mo for PNPLA8-high group; $P = 0.005$) (Figure 2). Therefore, higher PNPLA8 expression could predict poor overall survival in CRC patients, suggesting that PNPLA8 is a prognostic factor of CRC.

We then conducted further stratified analysis according to the TNM stage of CRC patients. For CRC patients at Stage I and II, PNPLA8 expression was a significant prognostic factor (HR 2.578, $P < 0.01$; Figure 3A). For CRC patients at Stage III, OS did not show statistical differences among patients with different PNPLA8 expression levels (HR 1.061, $P = 0.083$; Figure 3B). For CRC patients at Stage IV, patients with a higher PNPLA8 expression also had statistically significantly shorter OS (HR 1.476, $P = 0.036$; Figure 3C). In stratified analysis according to tumor location, PNPLA8 expression was not a significant prognostic factor for patients with right-sided colon cancer (Figure 3E) ($P = 0.7057$). However, for patients with left-sided colon (Figure 3D) and rectal cancer (Figure 3F), the survival curves of the two PNPLA8-expression groups showed statistically significant differences (HR 1.886, $P = 0.009$ for left-sided colon cancer; HR 1.583, $P = 0.035$ for rectal cancer).

PNPLA8 and several clinicopathological parameters are independent prognostic factors of CRC

Using univariate analysis, we found that CRC patients with PNPLA8-high expression showed significant differences compared to PNPLA8-low expression in terms of multiple parameters, including CEA ($P < 0.001$), CA199 ($P < 0.001$), primary differentiation ($P = 0.02$), T stage ($P < 0.001$), N stage ($P < 0.001$), M stage ($P < 0.001$), vascular invasion ($P < 0.001$), and nerve invasion ($P < 0.001$) (Table 3). Therefore, multivariate analysis was performed using the Cox proportional hazards model for all of the significant variables examined in the univariate analysis. We found that PNPLA8 expression was a statistically significant independent prognostic factor (HR 1.328, $P = 0.038$). In addition, CA199 (HR 1.548, $P = 0.004$), N stage (HR 1.701, $P < 0.001$), M stage (HR 4.862, $P < 0.001$) and vascular invasion (HR 1.512, $P = 0.017$) (Table 3) were also found to be independent factors.

DISCUSSION

In this study, a large patient cohort was evaluated for the prognostic value of PNPLA8 in CRC. Patients with a higher PNPLA8 expression had a significantly impaired OS. Moreover, PNPLA8 expression was identified as a new independent prognostic factor for OS of CRC patients.

PNPLA8 is part of a diverse family of phospholipase A2 enzymes (PLA_2s) that hydrolyze the *sn*-2 substituent from membrane phospholipids to release a free fatty acid and a lysolipid[16,11]. These enzymes are ubiquitously expressed, and in contrast to secretory PLA_2s and cytosolic PLA_2s, do not require Ca^{2+} for either translocation or activity. Some of the first descriptions of $iPLA_2$ activity were in the mid- to late-1980s with the identification of a plasmalogen-selective $iPLA_2$ in PNPLA8, when PNPLA8 was found to function as a phospholipase and was better characterized[17]. During the past few years, knockout and transgenic mice for manipulated PNPLA8 expression have been established[18], and studies using these gene-manipulated mice have provided models with which to elucidate the physiological and pathophysiological roles of PNPLA8. Recently, the mechanisms by which phospholipase A2 enzymes mediate lipid reprogramming and glycerophospholipid remodeling in cancer cells have been elucidated[19,20]. As the upstream regulators of the arachidonic acid cascade, PLA2s are generally highly expressed and activated in various cancers[19,20]. Therefore, they are potential pharmacological targets and biomarkers in cancer.

Table 3 Cox regression analyses for overall survival of colorectal cancer patients in this study

Variates	Overall survival			
	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value
PNPLA8		0.004		0.038
Low	1 (reference)		1 (reference)	
High	1.472 (1.132-1.914)		1.328 (1.016-1.734)	
Age in yr		0.561		
≤ 60	1 (reference)			
> 60	1.079 (0.835-1.394)			
Sex		0.087		0.903
Male	1 (reference)		1 (reference)	
Female	0.792(0.606-1.304)		0.903 (0.687-1.187)	
CEA in ng/mL		< 0.001		0.811
≤ 5	1 (reference)		1 (reference)	
> 5	2.126(1.636-2.763)		0.964 (0.715-1.300)	
CA199 in U/mL		< 0.001		0.004
≤ 37	1 (reference)		1 (reference)	
> 37	2.870 (2.191-3.759)		1.548 (1.150-2.083)	
Tumor location		0.050		0.895
Right-sided colon	1 (reference)		1 (reference)	
Left-sided colon	0.952 (0.687-1.320)		0.933 (0.667-1.305)	
Rectum	0.706 (0.518-0.962)		0.934 (0.680-1.284)	
Tumor size in cm		0.512		
≤ 4.0	1 (reference)			
> 4.0	1.090 (0.843-1.409)			
Primary histological type		0.371		
Non-mucinous	1 (reference)			
Mucinous	0.854 (0.603-1.208)			
Primary differentiation		0.002		0.162
Well/moderate	1 (reference)		1 (reference)	
Poor/anaplastic	1.507 (1.162-1.954)		1.210 (0.926-1.581)	
T stage		< 0.001		0.163
T1/T2	1 (reference)		1 (reference)	
T3/T4	3.058 (1.934-4.834)		1.415 (0.869-2.306)	
N stage		< 0.001		< 0.001
N0	1 (reference)		1 (reference)	
N1/N2	2.948 (2.852-3.859)		1.701 (1.272-2.274)	
M stage		< 0.001		< 0.001
M0	1 (reference)		1 (reference)	
M1	7.193 (5.520-9.372)		4.862 (3.608-6.551)	
Vascular invasion		< 0.001		0.017
No	1 (reference)		1 (reference)	

Yes	2.265 (1.649-3.111)		1.512 (1.078-2.121)	
Nerve invasion		< 0.001		0.218
No	1 (reference)		1 (reference)	
Yes	2.901 (1.986-4.236)		1.291 (0.860-1.939)	

CA199: Carbohydrate antigen 19-9; CEA: Carcinoembryonic antigen; HR: Hazard ratio; M: Presence of metastasis; N: Extent of tumor spread to the lymph nodes; PNPLA8: Patatin like phospholipase domain containing 8; T: Extent of the tumor (the size of the tumor and any spread of tumor into nearby tissue).

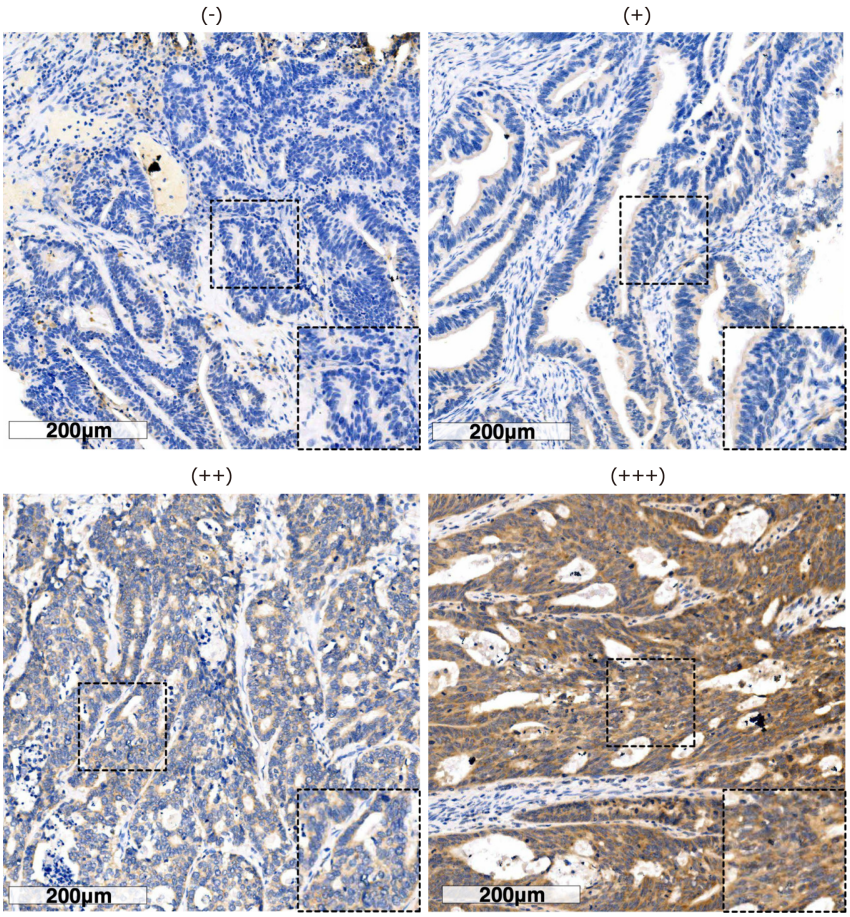

Figure 1 Immunohistochemical staining of patatin like phospholipase domain containing 8 protein in colorectal cancer specimens. Representative immunohistochemistry images show the staining intensities of patatin like phospholipase domain containing 8 protein, which were scored as 0 ("-"), 1 ("+"), 2 ("++"), and 3 ("+++"). Scale bar: 200 μm.

Our findings are in line with previous reports that PNPLA8 expression is increased in CRC[21,12]. The ability of PNPLA8 to promote cell proliferation becomes prominent in the context of tumorigenesis. Several *in vitro* studies revealed higher expression of PNPLA8 in stimulated immortal cell lines and that chemical inhibition or siRNA-mediated PNPLA8 knockdown reduces proliferation and promotes apoptosis[22,23]. Subsequent studies targeting specific cancers suggest that PNPLA8 promotes cancer cell growth *via* signal transduction pathways involving epidermal growth factor receptors, mitogen-activated protein kinases, E3 ubiquitin-protein ligase Mdm2, tumor suppressor protein p53, and cell cycle regulator p21[24,25]. Therefore, there is increasing support for a role of PNPLA8 and PNPLA8-associated phospholipase A in promoting cancer cell proliferation and metastasis, which plausibly provides the molecular mechanisms underlying our finding of PNPLA8 as a novel prognostic factor for CRC.

However, a couple of limitations of this study must be noted. First, our study is a retrospective one. To further validate our conclusion, a prospective study with data from multiple centers is necessary, especially for patients with left-sided colon and rectal cancer as well as Stage I and II patients. Second, the scores for PNPLA8 protein intensity and area were not determined in a fully automated way, resulting in potential artificial errors due to possible human bias. Third, in-depth *in vitro* and *in vivo* experiments are urgently needed to unveil the mechanisms of PNPLA8 in CRC.

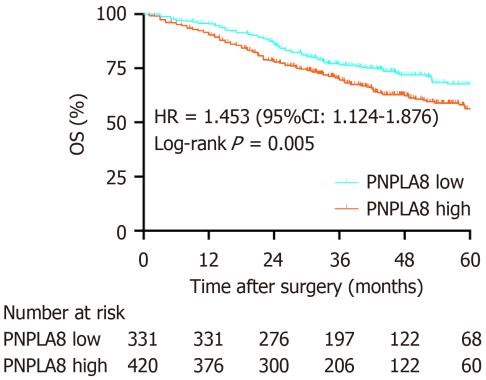

Figure 2 Kaplan-Meier analyses of overall survival rates in 751 colorectal cancer patients with a low or high patatin like phospholipase domain containing 8 protein expression. The colorectal cancer (CRC) patients with a high patatin like phospholipase domain containing 8 (PNPLA8) expression (n = 420) had an overall survival (OS) rate (P = 0.005) than those with a low PNPLA8 expression (n = 331) as determined using the Kaplan-Meier method.

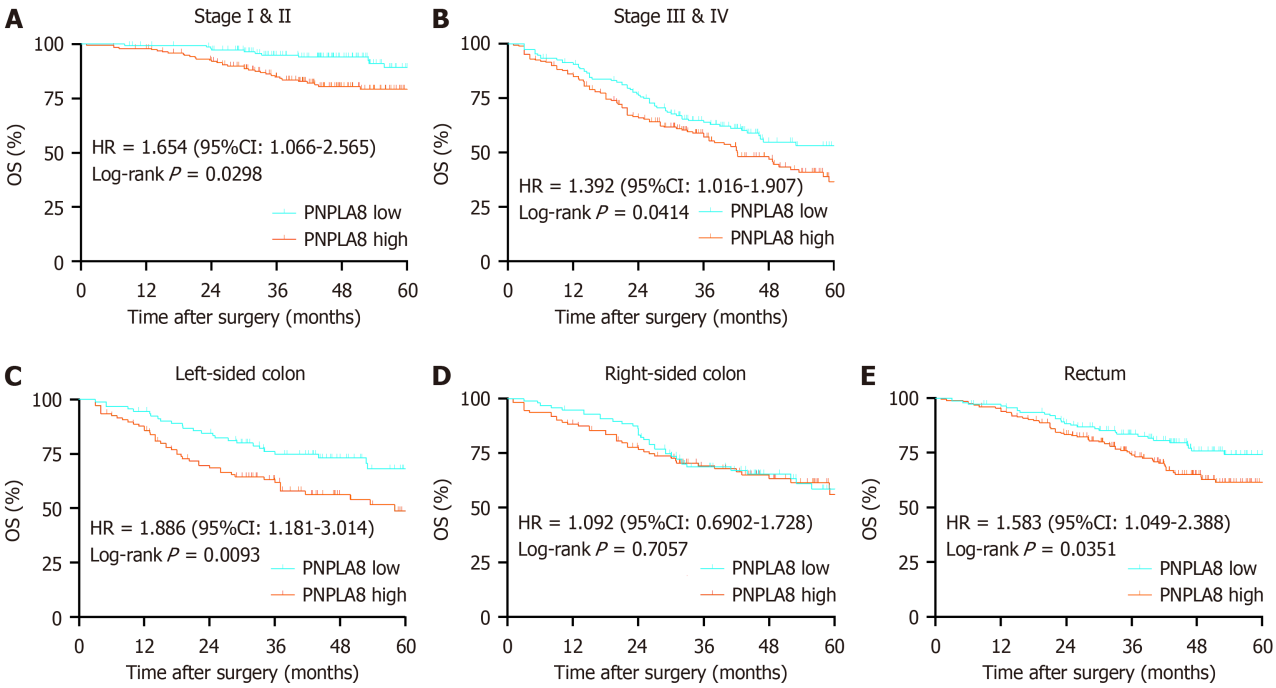

Figure 3 Patatin like phospholipase domain containing 8 protein expression according to TNM stage and tumor location. A: Patients at stage I and stage II [n = 153 for patatin like phospholipase domain containing 8 (PNPLA8) low group; n = 200 for PNPLA8 high group]; B: Patients at stage III and stage IV (n = 178 for PNPLA8 low group; n = 220 for PNPLA8 high group); C: Patients with left-sided colon cancer (n = 92 for PNPLA8 low group; n = 108 for PNPLA8 high group); D: Patients with right-sided colon cancer (n = 97 for PNPLA8 low group; n = 112 for PNPLA8 high group); E: Patients with rectal cancer (n = 142 for PNPLA8 low group; n = 200 for PNPLA8 high group). The hazard ratio (HR), 95% confidence interval (95%CI), and log-rank P values are indicated in each panel. OS: Overall survival.

CONCLUSION

In summary, PNPLA8 was identified as a new independent prognostic factor for CRC. CRC with high PNPLA8 expression conferred survival impairment. Our findings provide critical insights into aiding the individualized treatment of CRC patients.

ARTICLE HIGHLIGHTS

Research background

The role of patatin like phospholipase domain containing 8 (PNPLA8) in colorectal cancer (CRC) progression is unclear.

Research motivation
The research motivation is to explore the prognostic effects of PNPLA8 expression in CRC.

Research objectives
To evaluate the prognostic value of PNPLA8 expression level for CRC patient survival and its correlation with clinicopathological features of CRC patients.

Research methods
PNPLA8 expression in tumor samples was evaluated by immunohistochemistry staining and semi-quantitated with immunoreactive scores.

Research results
CRC patients with high PNPLA8 expression indicated poor overall survival (OS) (median OS = 35.3, P = 0.005). The multivariate analysis also confirmed that high PNPLA8 expression was a significantly independent prognostic factor for overall survival (hazard ratio HR = 1.328, 95%CI: 1.016-1.734, P = 0.038).

Research conclusions
High PNPLA8 expression level is associated with poorer survival outcomes in CRC patients, indicating its prognostic value for predicting patient outcomes.

Research perspectives
Further studies are needed to validate the prognostic value of PNPLA8 in multicenter cohorts of CRC patients. The mechanism of PNPLA8 in CRC development and progression remains to be fully investigated to help to identify poten-tial therapeutic targets and develop new treatment strategies.

FOOTNOTES

Author contributions: Zhou PY carried out the studies; Zhuang AB and Chen YJ participated in collecting data and drafted the manuscript; Mao YH and Feng QY performed statistical analysis and participated in its design; Zhu DX participated in acquisition, analysis, and interpretation of data and drafted the manuscript; all authors read and approved the final manuscript.

Institutional review board statement: This study was approved by the Clinical Research Ethics Committee of Zhongshan Hospital, Fudan University.

Informed consent statement: Informed consent was acquired from all patients of the primary cohort for the acquisition of clinical and pathological information and the use of surgical specimens.

Conflict-of-interest statement: The authors report having no relevant conflicts of interest for this article.

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