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Original Article

Prognostic significance of stromal SMYD3 expression in colorectal cancer of TNM stage I-III

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Abstract: Background: SET and MYND domain-containing protein 3 (SMYD3) is a histone methyltransferases and it promotes progression of many kinds of cancers including lung cancer, ovarian cancer and gastric cancer. In colorectal cancer (CRC), SMYD3 is proved to stimulate the proliferation of cancer cells, but the clinical significance of SMYD3 in CRC has not been elucidated. Methods: In our study, we detected the expression of SMYD3 in CRC samples in TNM stage I-III with immunohistochemistry. The correlation between the expression of SMYD3 and the clinicopathological factors was analyzed with Chi-square test. The survival curve was displayed by Kaplan-Meier test and the statistical difference of subgroups was analyzed with log-rank test. Independent prognostic factors were identified by the Cox proportional hazards regression model. Results: The percentage of high SMYD3 expression and low expression accounts for 47.98% and 52.02% respectively. High expression of SMYD3 was significantly associated with advance T stage (P=0.006) and lower survival rates (P=0.010), and it could be identified as an independent prognostic factor indicating unfavorable prognosis of patients with CRC (P=0.032, HR=1.98, 95% CI=1.06-3.70). Conclusions: SMYD3 high-expression is a high risk for poorer prognosis of CRC in TNM stage I-III. Our findings suggested that detecting SMYD3 may help stratify patients by risk more preciously and help make the individual treatment strategy.

Keywords: SET and MYND domain-containing protein 3, colorectal cancer, tumor invasion, prognosis

Introduction

Colorectal cancer (CRC) is the third most common cause of cancer deaths with about 13 million new cancer cases and 694,000 cancer deaths in 2012 worldwide [1, 2]. In developing countries like China, the incidence of CRC is increasing significantly. Major high risk factors of CRC include genetic and environmental factors, a heat-treated high-fat diet, obesity, and a sedentary lifestyle [3]. Although the diagnosis and treatment equipment have developed rapidly in recent years, the 5-year overall survival rate of CRC remains about 30% in China [4, 5]. The reasons contributing to the unsatisfactory prognosis of CRC are its late tumor presentation, rapid progression and high recurrence [6]. About 20% of these patients suffer from tumor recurrence and metastasis even after radical surgery and systemic adjuvant chemotherapy [7]. So effective prognostic biomarkers are urgently needed to predict the prognosis and define the high-risk patients for adjuvant chemotherapy.

SET and MYND domain-containing protein 3 (SMYD3) is a histone methyltransferases which methylates 'Lys-4' of histone H3 and induces di- and tri-methylation instead of monomethylation [8, 9]. The Smyd-family consists of 5 members (SMYD1, 2, 3, 4 and 5), containing a SET domain methyltransferases and an intervening MYND domain. The SET domain is essential to promote the dimethylation or trimethylation of chromosomal histone, resulting in a loosened state of the chromosome's spatial structure and affecting gene transcription [8, 10]. The MYND domain can enhance the methylation function of the SET domain by mediating protein-protein interactions [11, 12]. Overexpression of SMYD3 has been reported to be involved in the progression or prognosis in many kinds of cancers, including gastric cancer, leukemia, ovarian cancer, and so on [13,

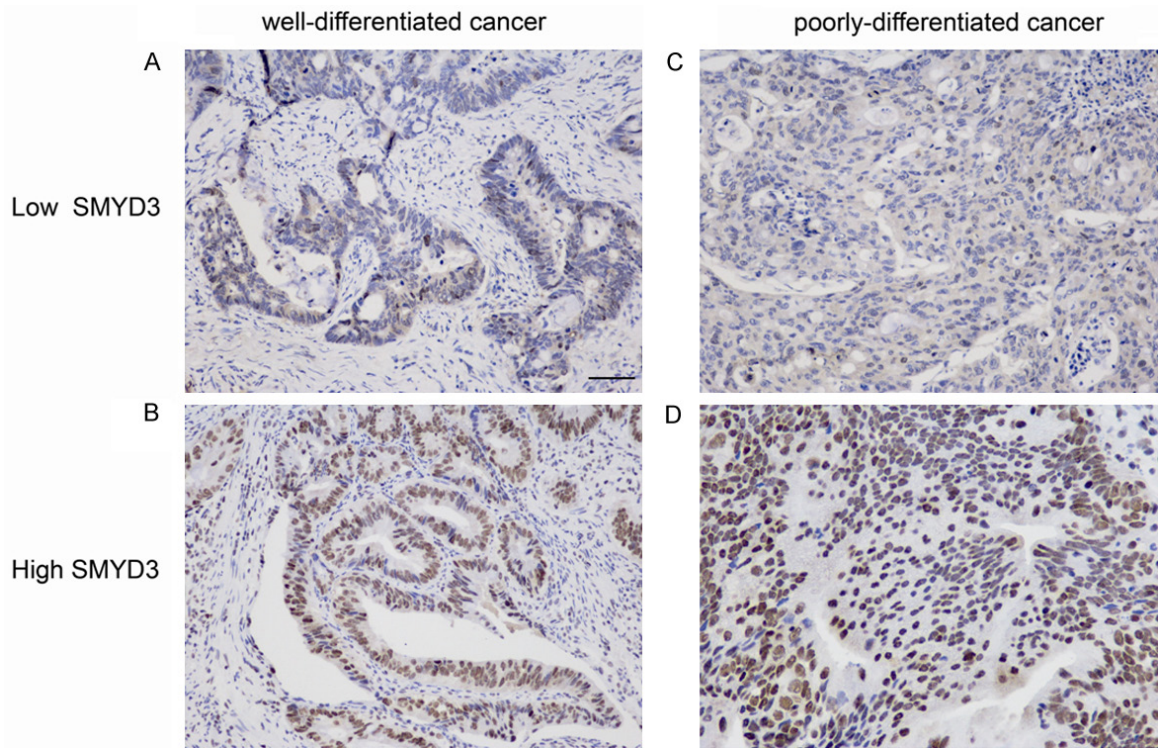

Figure 1. Representative immunohistochemical figure. The representative image for low-expression or high-expression of SMYD3 in well-differentiated (A and B) and poorly-differentiated (C and D) colon cancer. Scale bar: 100 μm.

14]. It is demonstrated that SMYD3 is an oncoprotein via functioning as a transcriptional potentiator of multiple cancer-promoting genes, by which SMYD3 promotes cell proliferation and epithelial-mesenchymal transition in colon cancer [15]. However, the clinical significance of SMYD3 in CRC is still unknown.

In our study, we detected the expression of SMYD3 in CRC samples in TNM stage I-III with immunohistochemistry (IHC), and analyzed the correlation between SMYD3 expression, clinicopathological factors and overall survival rates. Moreover, we identified the independent prognostic factors of CRC.

Materials and methods

Patients and follow-up

Our study was approved by the committee of Linyi Central Hospital. A total of 173 patients diagnosed as CRC from 2004 to 2010 were selected into our cohort according to the criteria (1) patients were in TNM stage I-III and underwent radical surgical resection, (2) available follow-ups more than 5 months and enough specimens for IHC, (3) the TNM stage of CRC was in stage I to III. All the specimens were obtained from the Department of Pathology with prior consents of patients. The diagnosis of CRC was double confirmed by two senior pathologists, and the TNM stage in our study was according to the 7th American Joint Committee on Cancer/Union for International Cancer Control.

Immunohistochemistry

The expression of SMYD3 was detected with IHC described previously in detail [16, 17]. The formalin-fixed and paraffin-embedded specimens were first deparaffinized at 55°C for 20 minutes and then washed with xylene. Rehydration of samples was realized by graded ethanol with concentration at 100%, 95%, and 80%. Slides were incubated in 3% hydrogen peroxide to block the endogenous peroxidase activity and in citrate buffer (pH=6.0) with a microwave oven for optimal antigen retrieval. Followed by blocking unspecific binding with incubation in 5% bovine serum albumin for 30 minutes, primary antibody of SMYD3 (#12,859, 1:500 dilution; Cell Signaling Technology, Danvers, MA,

Table 1. Basic information of patients

Characters	Number	Percentage
Sex		
Male	81	46.82%
Female	92	53.18%
Age		
<60	72	41.62%
≥60	101	58.38%
Tumor diameter (cm)		
≤5	69	39.88%
>5	104	60.12%
T stage		
T1+T2	77	44.51%
T3+T4	96	55.49%
Lymph node invasion		
No (N0)	105	60.69%
Yes (N1/2)	68	39.31%
TNM stage		
I	50	28.90%
II	55	31.79%
III	68	39.31%
Differentiation		
Good	97	56.07%
Moderate	48	27.75%
Poor	28	16.18%
Adjuvant therapy		
No	54	31.21%
Yes	112	64.74%
SMYD3		
Low	90	52.02%
High	83	47.98%

Abbreviations: SMYD3 = SET and MYND domain-containing protein 3.

USA) was used to incubate the specimen at 4°C overnight. After rinsing the slide with phosphate buffered saline for 3 times, the biotin-labeled secondary antibody (Beyotime Institute of Biotechnology, Shanghai, China) and streptavidin-peroxidase (Beyotime Institute of Biotechnology, Shanghai, China) were used to incubate the specimen. The visualization of slides was achieved by interaction with 3,3'-diaminobenzidine substrate.

Evaluation of IHC results

The results of IHC were blindly evaluated by two senior pathologists unaware of the clinical data. According to previous reports [18], the score system of IHC consists of two parts: the score of IHC positive cell percentage and the score of staining intensity. The score of IHC positive cell percentage was defined as follows: 1, less than 25% of positive cells; 2, 25%-50% of positive cells; 3, 50%-75% of positive cells; and 4, more than 75% of positive cells. The score of staining intensity was described as: 0 for negative staining, 1 for weak staining, 2 for moderate staining, and 3 for strong staining. The receiver operating characteristic (ROC) curve was fitted to identify the point with the highest sum of sensitivity and specificity, which was defined as the cut-off of IHC score [19]. The cut-off divided the patients into high- and low-expression of SMYD3.

Table 2. Correlations between clinicopathological factors and SMYD3 expression

Characters	SMYD3		P*
	Low	High	
Sex			
Male	41	40	0.728
Female	49	43	
Age			
<60	31	41	0.046
≥60	59	42	
Tumor diameter (cm)			
≤5	41	28	0.112
>5	49	55	
T stage			
T1+T2	49	28	0.006
T3+T4	41	55	
Lymph node invasion			
No (N0)	59	46	0.173
Yes (N1/2)	31	37	
TNM stage			
I	33	17	0.060
II	26	29	
III	31	37	
Differentiation			
Good	44	53	0.140
Moderate	29	19	
Poor	17	11	

*means calculated by χ^2 test. Abbreviations: SMYD3 = SET and MYND domain-containing protein 3.

Statistical analysis

All data were analyzed with software SPSS 22.0 (IBM cooperation, USA). The correlation

Table 3. Correlation between SMYD3 and clinicopathological factors

Characters	5-year survival rate	P*
Sex		
Male	71.8	0.414
Female	61.1	
Age		
<60	59.5	0.574
≥60	70.8	
Tumor diameter (cm)		
≤5	72.5	0.312
>5	58.8	
T stage		
T1+T2	78.9	0.003
T3+T4	52.5	
Lymph node invasion		
No (N0)	74.0	0.008
Yes (N1/2)	54.7	
TNM stage		
I	87.6	0.002
II	56.5	
III	54.7	
Differentiation		
Good	70.4	0.059
Moderate	66.6	
Poor	50.6	
Adjuvant therapy		
No	44.0	0.015
Yes	73.2	
SMYD3		
Low	75.8	0.010
High	50.4	

*means calculated by log-rank test. Abbreviations: SMYD3 = SET and MYND domain-containing protein 3.

between the expression of SMYD3 and the clinicopathological factors was analyzed with Chi-square test. The survival curve was displayed by Kaplan-Meier test and the statistical difference of subgroups was analyzed with log-rank test. Independent prognostic factors were identified by the Cox proportional hazards regression model. P<0.05 was considered as statistically significant.

Results

Expression of SMYD3 in CRC tissues

The expression and location of SMYD3 in CRC tissues was explored by the IHC. As a histone methyltransferases, SMYD3 was mainly observed in the nucleus in our experiment, which is corresponding to its function. According to the expression of SMYD3, the cohort was divided into subgroups of high SMYD3 expression and low expression. The representative images for SMYD3 low-expression group and high-expression were displayed in **Figure 1A** and **1B**. The percentage of high SMYD3 expression and low expression accounts for 47.98% and 52.02% respectively (**Table 1**).

Correlation between SMYD3 and clinicopathological factors of CRC

The χ^2 test was performed to analyze the correlation between SMYD3 expression and clinicopathological factors of CRC (**Table 2**). In our study, the T stage was significantly associated SMYD3 expression (P=0.006). Patients with high SMYD3 expression had a more possibility to have advanced T stage compared with the patients with low SMYD3 expression. Similarly, advanced TNM stage tended to be substantially associated with high expression of SMYD3, but the statistical significance was not so remarkable (P=0.060). Intriguingly, younger patients (<60 years old) seemed to have a change to have high expression of SMYD3, which might be partially because of the higher metabolism requiring SMYD3 function.

Association between SMYD3 expression and the survival rates

The univariate analysis was first carried out to examine the significant factors associated with the overall survival rates (**Table 3**). In our experiments, high expression of SMYD3 was significantly associated with lower survival rates (P=0.010). The 5 year overall survival rates of high expression and low expression of SMYD3 were 50.4% and 75.8% (**Figure 2**). Moreover, advanced T stage and TNM stage, positive lymphatic invasion and no adjuvant therapy were all proved to be remarkably associated with the overall survival rates of TNM I-III CRC (P=0.003, 0.002, 0.008 and 0.015, respectively). Levels of tumor differentiation also seemed to be a prognostic factor with a statistical insignificant value (P=0.060).

Independent prognostic factor of CRC

The multivariate analysis was also performed to identify the independent prognostic factors

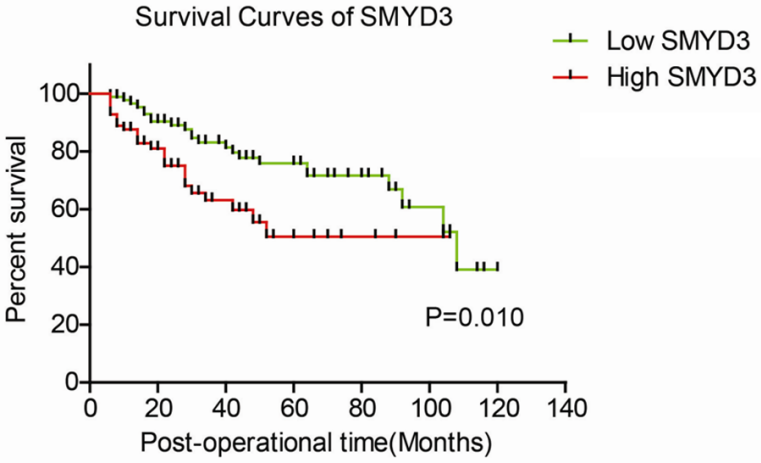

Figure 2. Overall survival curve of SMYD3. The overall survival curve of low-expression and high-expression of SMYD3 was drawn with Kaplan-Meier method and the statistical difference was calculated by log-rank test. Patients with high SMYD3 expression had significantly low survival rates compared with those with low expression of SMYD3.

Table 4. Multivariate analysis

Characters	HR	95% CI	P*
T stage			
T1+T2	1		
T3+T4	2.23	1.17-4.24	0.015
Lymph node invasion			
No (N0)	1		
Yes (N1/2)	1.65	0.92-2.97	0.096
Differentiation			
Good	1		
Moderate	1.44	0.72-2.88	0.308
Poor	3.04	1.41-6.56	0.005
Adjuvant therapy			
Yes	1		
No	2.23	1.19-4.17	0.012
SMYD3			
Low	1		
High	1.98	1.06-3.70	0.032

*means calculated by Cox-regression model. Abbreviations: SMYD3 = SET and MYND domain-containing protein 3. HR = hazard ratio, 95% CI = 95% confidence interval.

of CRC. All the defined prognostic parameters were enrolled into the Cox-regression hazard model, and tumor differentiation was also selected because of its tendency to affect the prognosis (**Table 4**). TNM stage was excluded because it had obvious interaction with T stage and lymphatic status. High expression of SMYD3 was identified to be an independent prognostic factor of CRC in TNM stage I-III (P=0.032, HR= 1.98, 95% CI=1.06-3.70). Additionally, the advanced T stage, poor differentiation and no adjuvant therapy was confirmed to be high risk for prognosis of patients with CRC (P=0.015, 0.005 and 0.032, respectively).

Discussion

According previous studies, SMYD3 could promote cancer progression by activating the transcription of multiple target genes via making chromatin more accessible via catalyzing histone methylation, or interacting the promoter of target genes and initiating transcription by associating with RNA polymerase II and RNA helicase [15, 20]. In carcinogenesis and cancer progression, there are many genes regulated by SMYD3. Many downstream genes of SMYD3 have been discovered including 15-LOX-1, Nkx2.8, RIZ1, c-Met, WNT10B, MMP9, and androgen receptor [21-23]. Their transcription was regulated by the function and expression of SMYD3. On the contrary, SMYD3 expression was also reported to be elevated in presence of KRAS mutation in several kinds of cancers such as rectal cancer and lung adenocarcinoma [24]. In CRC, previous studies demonstrated that SMYD3 could promote the cell proliferation of CRC cell lines [8]. In our study, we demonstrated that SMYD3 overexpression was an independent biomarker indicating poorer prognosis of CRC. The genes and proteins regulated by SMYD3 and the underlying mechanisms are still unknown. We hope more interests could be focused on SMYD3 onco-function in CRC, helping explore the signaling network downstream of SMYD3.

CRC is still the leading cause of cancer deaths in developed countries. The application of adjuvant therapies like chemotherapy increases the overall survival rate of patients with CRC remarkably. More effective drugs especially target drugs are still in urgent need for the treatment of CRC. The aberrant SMYD3 level could regulate the epigenetic transcription of target genes and exhibit the tumorigenic effects, mak-

ing SMYD3 a hotspot in cancer treatment. Based on this oncogenic function of SMYD3, it is considered as a drug target for novel anticancer agents [25]. The inhibitor of SMYD3 such as BCI-121 has been demonstrated to impair cell growth of SMYD3-overexpressing solid cancer [26]. Several other compounds like EPZ030456 or GSK2807 were also proved to inhibit the SMYD3 catalytic activity with experiments in vitro. However, more animal experiments or clinical trials should be carried out to push the proceeding of SMYD3 to be the drug target.

In summary, we detected the expression of SMYD3 in 173 patients with CRC in TNM stage I-III, and demonstrated SMYD3 high-expression to be a high risk for poorer prognosis of CRC. Our findings suggested that detecting SMYD3 may help stratify patients by risk more preciously and help make the individual treatment strategy.

Disclosure of conflict of interest

None.

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