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Original Article

Overexpression of SOX9 and DNMT1 predicts poor prognosis and chemoresistance of colorectal cancer

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Abstract: Colorectal cancer is one of the most leading causes of cancer-related death. Epithelial-mesenchymal transition (EMT) is integral in cancer stem cell behavior, and contributes pathologically to cancer progression. Both SOX9 and DNMT1 have predominant roles in EMT. The purpose of this study is to determine the expression pattern and clinical relevance of SOX9 and DNMT1 in human CRC. Immunohistochemical staining showed that SOX9 and DNMT1 overactivated in CRC (P<0.001, P<0.001). SOX9 (hi) expression was positively associated with histological classification of CRC patients (P=0.001). DNMT1 (hi) expression was significantly associated with histological classification (P<0.001), LN stages (P<0.001) and TNM stages (P=0.001) of CRC patients. SOX9 expression was positively correlated with DNMT1 expression (ρ=0.540, P<0.001). SOX9 (hi) DNMT1 (hi) expression was positively associated with histological classification (P=0.003), LN stages (P=0.018) and TNM stages (P=0.004) of CRC patients. In 184 patients with survival information, both SOX9 (hi) and DNMT1 (hi) expressions were associated with unfavorable prognosis (P<0.001, P<0.001). SOX9 (hi) DNMT1 (hi) expression patient shown much poorer prognosis (P<0.001). Cox regression analysis showed that histological classification, LN stage, SOX9 (hi) expression, DNMT1 (hi) expression and double (hi) expression of SOX9 and DNMT1 were independent prognostic factors. This study provides the first evidence that SOX9 and DNMT1 are both relevant to malignant characteristics of CRC. Our data suggests the potential of SOX9 and DNMT1 as prognostic biomarkers for CRC and predictive marker of FOLFOX regimens.

Keywords: SOX9 DNMT1 colorectal cancer prognosis

Introduction

Colorectal cancer has been the third most common cancer and the fourth cause of cancer-related death worldwide [1]. The prognosis of patients with colorectal cancer has slowly but steadily improved because of improved early detection and treatment [1]. 5-year relative survival has reached almost 65% in developed countries, such as Australia, Canada, the USA, and several European countries, but has remained less than 50% in developing countries [1]. Chemoresistance and metastasis are the main causes of treatment failure [2]. Epithelial-mesenchymal transition (EMT) is a crucial process in embryonic development that allows epithelial cells to lose apical-basal polarity and cell-cell contacts while gaining mesenchymal phenotypes [3, 4]. And it is utilized by cancer cells to gain stem-like properties, such as enhanced survival, self-renewal, and anchorage-independent growth, all of which contribute to increased metastasis and survival under the stress, such as drug-treatment or radiotherapy [3, 4]. Indeed, EMT-related markers, such as EMT transcription factors are predictive for increased invasion, loss of differentiated characteristics, metastasis, and poor prognosis in a number of human tumor types [4].

Sex-determining region Y (SRY) box 9 (SOX9) is a member of EMT-transcription factors, which are activated in stem cells and essential for organs development [5]. For example, SOX9 was activated during neural stem cells (NSCs) specification, while loss of SOX9 led ependymal cells to adopt a neuroblast identity, which indicates SOX9 is essential for NSCs maintenance

Table 1. Clinicopathologic characteristics of 184 CRC cases

Clinicopathologic characteristics	Case
Median age	64 (30, 88)
Gender	
Male	86
Female	98
Location	
Colon	90
Rectum	94
T stage	
T1	3
T2	31
T3	89
T4	61
N stage	
N0	97
N1	56
N2	31
TNM	
I	26
II	71
III	87
Histological grade	
I	18
II	132
III	34
Death	
Yes	48
No	136

[6]. Ectopic expression SOX9 with oncogenes, such as RAS, or other EMT-TFs, such as SLUG transformed the terminally differentiated cells to stem cells, and initiated tumorigenesis in breast and pancreas [7, 8]. And SOX9 overexpression has been found in many types of cancer, including skin, prostate, lung, brain, colon, ovary, pancreas, prostate, lung, peripheral nerves, and brain [5, 9-11]. Lü B, et al found that SOX9 was overexpressed in colorectal cancer and that overexpression of SOX9 predicted poor prognosis [12]. However, the role about the expression of SOX9 of CRC in chemoresistance is still unclear.

During epithelial-mesenchymal transitions, widespread changes of gene expression has been observed [3]. Epigenetic modification, including histone methylation and acetylation, and DNA methylation, is the main regulatory mechanism [8]. DNMT1 is the major DNA methyltransferase in mammalian cells, which is responsible for greater than 95% gene regulation during somatic cell development and differentiation, and is essential for survival of the cells [13, 14]. Moreover, DNMT1 overexpression are observed in many malignancies [15]. And overexpression of DNMT1 in colorectal cancer is associated with the malignant phenotype, such as poor differentiation [16]. Also, it is observed that DNMT1 is associated with chemoresistance in solid tumors [17]. However, the role about the expression of DNMT1 of CRC in chemoresistance is still unclear.

It is hypothesized that components that promotion of EMT and maintenance of mesenchymal status may be associate with unfavorable prognosis. To test this hypothesis, we investigated the EMT-TF SOX9 and EMT epigenetic regulatory enzyme DNMT1 expression in 184 CRC samples by IHC and explored the connection between SOX9/DNMT1 expression and CRC characteristics and evaluated their potential relation to the clinical outcome.

Materials and methods

Human samples

A total of 184 CRC samples were obtained from the Affiliated Hospital of Jiangnan University, which were collected between January 2006 and December 2008. Follow-up was monitored until December 2013, with a median follow-up of 65.7 months. All of the patients received the treatment of FOLFOX4 regimen [5-fluorouracil (5-Fu) with Oxaliplatin (OXA)] after surgery, according to the temporal NCCN guideline. The overall survival (OS) time was defined as the interval between the dates of surgery and death. The detailed clinicopathological features are listed in **Table 1.** The study was approved by the Research Ethics Committee of the Affiliated Hospital of Jiangnan University.

Immunohistochemical staining

Immunohistochemical staining (IHC) was used to determine the level of protein expression in situ. The paraffin-embedded sections of CRC tissues were deparaffinized, rehydrated, antigen retrieval and then incubated with the primary antibodies, anti-SOX9 (1:100) and anti-DNMT1 (1:100) at 4°C overnight. After that,

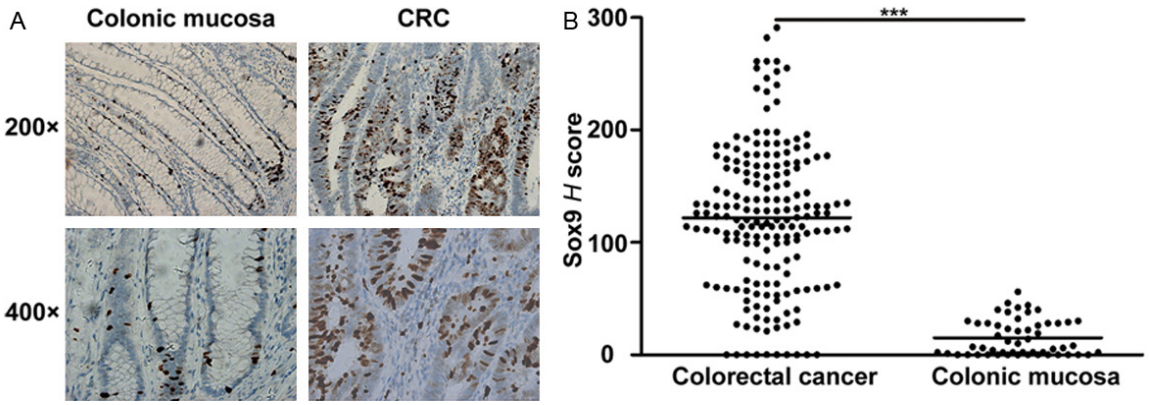

Figure 1. IHC analysis of SOX9 in CRC. A. Representative images of Sox9 expression at different magnification in sections from human CRC (n=184) and normal colonic mucosa (n=54). B. H score of Sox9 immunohistochemical staining for human CRC (121.9±64.4, n=184) and normal colonic mucosa (15.15±16.02, n=54).

Table 2. Associations between SOX9 expression and clinicopathological characteristics in CRC

Clinicopathologic characteristics	SOX9 expression		χ^2	*P*
	high	low		
Age (years)				
>64	47	44	0.09	0.767
≤64	46	47		
Gender				
Male	36	50	4.87	0.027
Female	57	41		
Location				
Colon	44	46	0.19	0.660
Rectum	49	45		
T stage				
T1	1	2	2.05	0.562
T2	16	16		
T3	41	47		
T4	35	26		
N stage				
N0	42	55	4.45	0.108
N1	32	24		
N2	19	12		
TNM				
I	9	17	5.38	0.068
II	33	38		
III	51	36		
Histological grade				
I	6	12	14.84	0.001
II	60	72		
III	27	7		
Death				
Yes	36	12	15.54	<0.01
No	57	79		

primary antibody was removed and washed, and then incubated with peroxidase-conjugated secondary antibody for 15 min each. Next, the slides were counterstained with hematoxylin using DAB Horseradish Peroxidase Color Development Kit (Boster Co., Wuhan, China) and assessed microscopically for positive DAB staining.

The immunostaining results were assessed by two observers independently. SOX9 and DNMT1 expression in nuclei of epithelial cells was considered as positive staining. Semiquantitative expression of SOX9 and DNMT1 was based on staining intensity and distribution using an H score system. The staining intensity was graded as follows: 0 (negative), 1 (weak), 2 (medium) and 3 (strong). The percentage of positive-staining cells was recorded as follows: a (percentage of weak positive-staining cells), b (percentage of medium positive-staining cells), and c (percentage of strong positive-staining cells) according to the percentages of the positive-staining areas in relation to the whole carcinoma area. H score = 1×a + 2×b + 3×c. Then, patients were separated into two groups according to their H score (hi: H score > median, lo: H score < median).

Statistical analysis

Data were analyzed using SPSS 21.0 statistical software (SPSS, Chicago, IL, USA). Categorical variables were compared using the Fisher's exact test or chi-square test with Yates correction. The survival time was evaluated using the

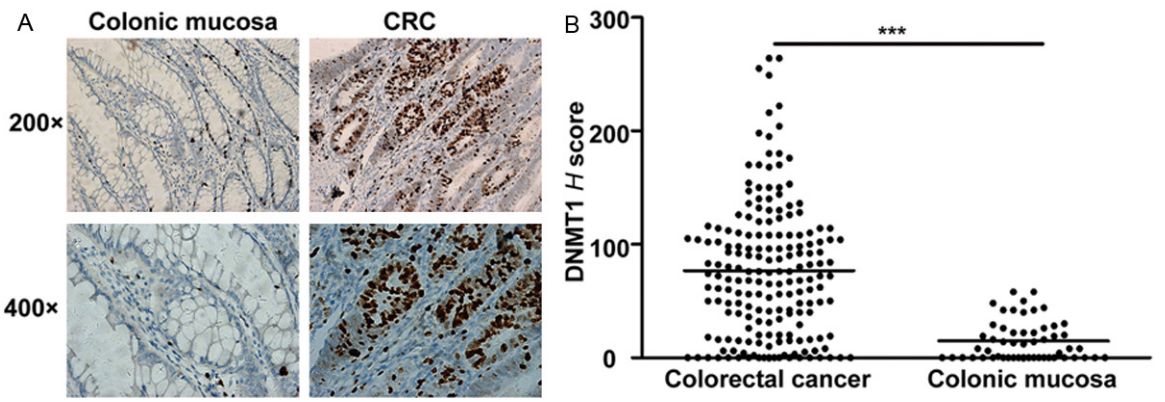

Figure 2. IHC analysis of DNMT1 in CRC. A. Representative images of DNMT1 expression at different magnification in sections from human CRC (n=184) and normal colonic mucosa (n=54). B. H score of DNMT1 immunohistochemical staining for human CRC (77.1±59.2.4, n=184) and normal colonic mucosa (14.94±17.49, n=54).

Table 3. Associations between DNMT1 expression and clinicopathological characteristics in CRC

Clinicopathologic characteristics	DNMT1 expression		χ^2	*P*
	high	low		
Age (years)				
>64	51	40	1.77	0.183
≤64	43	50		
Gender				
Male	40	46	1.35	0.245
Female	54	44		
Location				
Colon	51	39	2.20	0.138
Rectum	43	51		
T stage				
T1	1	2	5.98	0.112
T2	12	20		
T3	43	45		
T4	38	23		
N stage				
N0	37	60	15.20	<0.01
N1	34	22		
N2	23	8		
TNM				
I	7	19	15.54	<0.01
II	30	41		
III	57	30		
Histological grade				
I	6	12	14.44	0.001
II	61	71		
III	27	7		
Death				
Yes	38	10	20.49	<0.01
No	56	80		

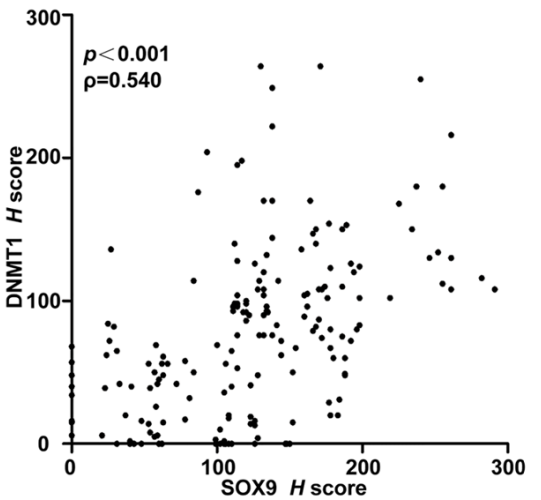

Figure 3. Correlation of SOX9 and DNMT1 expression in CRC tissues. The expression of proteins was detected by immunohistochemical staining. The graph showing the correlation between DNMT1 H score and those of SOX9 in CRC samples. A Spearman's test was used, and the correlation coefficient (ρ) and the two-tailed significance are shown.

Kaplan-Meier's method and analyzed using log-rank tests. Univariate and multivariate analyses were performed with Cox proportional hazards regression model. Associations of two parameters were given by spearman rank analysis. Receiver operating characteristic (ROC) curve analysis was applied to assess the predictive values of variables. A *P* value less than 0.05 was considered statistically significant. *, ** and *** indicate statistical significance relative to $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

Table 4. Associations between SOX9 and DNMT1 expression and clinicopathological characteristics in CRC

Clinicopathologic characteristics	SOX9 and DNMT1 expression		χ^2	*P*
	SOX9 (hi) DNMT1 (hi)	Others		
Age (years)				
>64	38	53	1.393	0.238
≤64	31	62		
Gender				
Male	28	58	1.683	0.195
Female	41	57		
Location				
Colon	37	53	0.980	0.322
Rectum	32	62		
T stage				
T1	0	3	6.606	0.086
T2	8	24		
T3	32	56		
T4	29	32		
N stage				
N0	28	69	7.981	0.018
N1	29	27		
N2	12	19		
TNM				
I	3	23	11.075	0.004
II	25	46		
III	41	46		
Histological grade				
I	4	14	11.312	0.003
II	44	88		
III	21	13		
Death				
Yes	27	21	9.741	0.002
No	42	94		

Results

SOX9 expression in CRC

To explore the SOX9 expression in CRC, we analyzed SOX9 expression in CRC and non-tumor colonic mucosa (NTC) by IHC. As shown in **Figure 1A**, SOX9 expressed in both NTC and CRC tissues. However, semiquantitative analysis showed that SOX9 was overexpressed in CRC (H score: 121.9±64.4 vs 15.15±16.02; CRC vs NTC, *P*<0.001, **Figure 1B**). As shown in **Table 2**, the levels of SOX9 in tumor tissues were positively associated with histological classification of CRC patients (*P*=0.001).

DNMT1 expression in CRC

To explore the DNMT1 expression in CRC, we also analyzed DNMT1 expression in CRC and non-tumor colonic mucosa (NTC) by IHC. As shown in **Figure 2A**, DNMT1 expressed in both NTC and CRC tissues. However, semiquantitative analysis showed that DNMT1 was overexpressed in CRC (H score: 77.1±59.2 vs 14.94±17.49; CRC vs NTC: *P*<0.001, **Figure 2B**). As shown in **Table 3**, the levels of DNMT1 in tumor tissues were positively associated with histological classification (*P*<0.001), LN stages (*P*<0.001) and TNM stages (*P*=0.001) of CRC patients.

Interrelationship of SOX9 expression and DNMT1 expression in CRC

As is well-known, both SOX9 and DNMT1 are important EMT-related genes. However, the relationship of SOX9 and DNMT1 is unknown. To reveal the relationship of SOX9 and DNMT1, we analyzed H score of SOX9 and DNMT1 in 184 CRC tissues by spearman correlation analysis. As shown in **Figure 3**, a significant positive association of SOX9 expression and DNMT1 expression was observed in CRC (ρ=0.540, *P*<0.001). As SOX9 expression is positively associated with DNMT1 expression in CRC, we explored the clinical relevance of both SOX9 (hi) and DNMT1 (hi) expression. As shown in **Table 4**, double high expression SOX9 and DNMT1 [SOX9 (hi) DNMT1 (hi)] in tumor tissues were positively associated with histological classification (*P*=0.003), LN stages (*P*=0.018) and TNM stages (*P*=0.004) of CRC patients.

Survival analysis

In 184 CRC patients with outcome information, the 5-year survival rate was 73.91% and the median survival time was 26.9±2.9 months (**Figure 4A**). To analyze the impact on survival of SOX9 and DNMT1 expression, Kaplan–Meir analysis and log-rank test were used. As shown

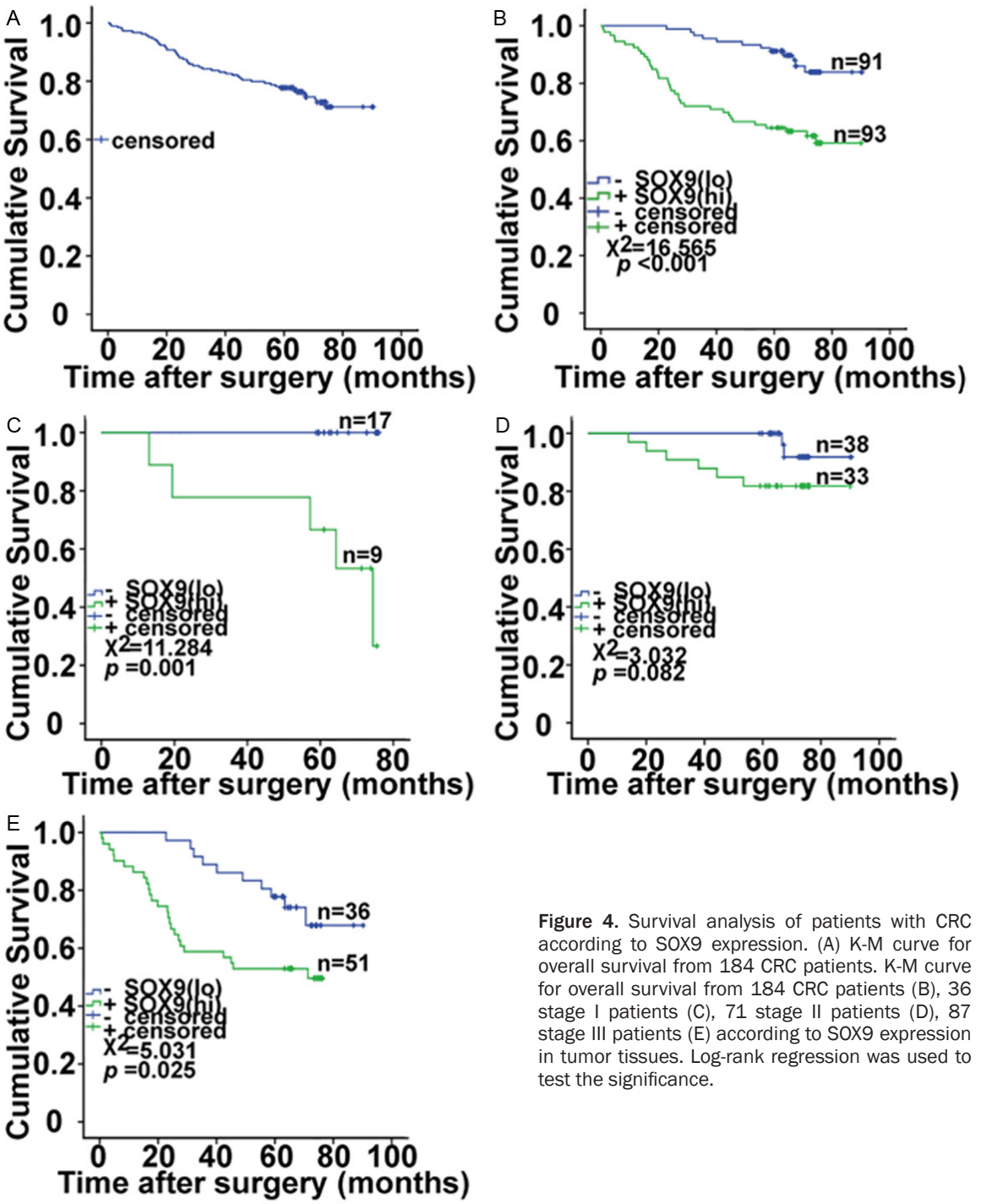

Figure 4. Survival analysis of patients with CRC according to SOX9 expression. (A) K-M curve for overall survival from 184 CRC patients. K-M curve for overall survival from 184 CRC patients (B), 36 stage I patients (C), 71 stage II patients (D), 87 stage III patients (E) according to SOX9 expression in tumor tissues. Log-rank regression was used to test the significance.

in **Figure 4B**, SOX9 (hi) expression was associated with a poor prognosis. The median survival time of SOX9 (hi) expression was 23.2±3.1 months, and the median survival time of Sox9 (lo) expression was 48.9±13.3 months. The log-rank test showed the expression of SOX9 protein in CRC was correlated significantly with patients' survival time (χ^2=16.565, P<0.001). Furthermore, subgroup analysis of each TNM stage shown only SOX9 (hi) expression in patients of stage I and III was associated with poor prognosis (**Figure 4C-E**).

As shown in **Figure 5A**, DNMT1 (hi) expression was associated with a poor prognosis. The median survival time of DNMT1 (hi) expression

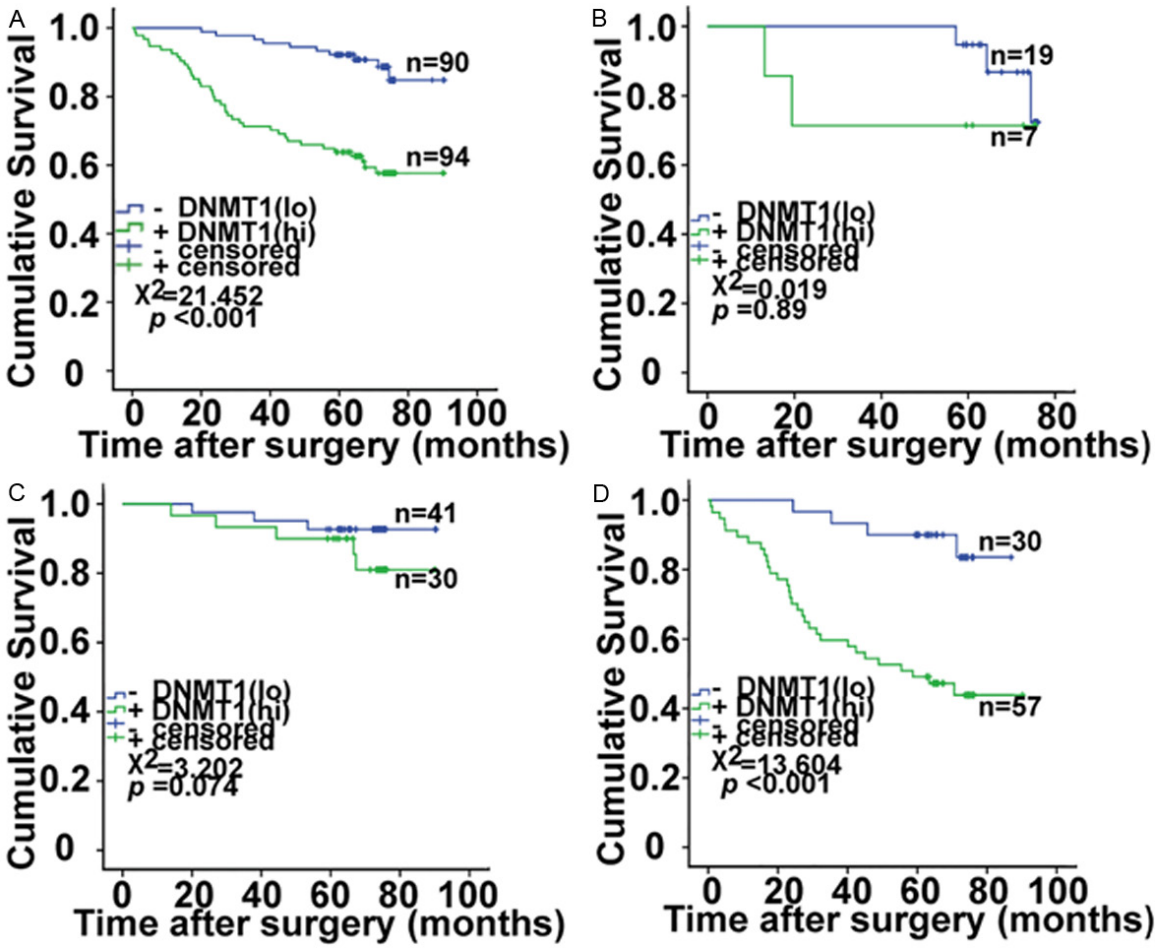

Figure 5. Survival analysis of patients with CRC according to DNMT1 expression. K-M curves for overall survival from 184 CRC patients (A), 36 stage I patients (B), 71 stage II patients (C), 87 stage III patients (D) according to DNMT1 expression in tumor tissues. Log-rank regression was used to test the significance.

was 23.5±2.2 months, and the median survival time of DNMT1 (lo) expression was 45.7±12.3 months. The log-rank test showed the expression of DNMT1 protein in CRC was correlated significantly with patients' survival time (χ^2=21.452, *P*<0.001). Furthermore, subgroup analysis of each TNM stage shown only SOX9 (hi) expression in patients of stage III was associated with poor prognosis (**Figure 5B-D**).

Due to the positive relationship between SOX9 expression and DNMT1 expression, we also analyzed the effect of both SOX9 (hi) and DNMT1 (hi) expression on CRC survival. As shown in **Figure 6A**, SOX9 (hi) DNMT1 (hi) expression was associated with a poor prognosis. The median survival time of SOX9 (hi) DNMT1 (hi) expression was 17.7±2.3 months, and the median survival time of the other expression was 53.4±7.4 months. The log-rank test showed the expression of SOX9 and DNMT1 protein in CRC was correlated significantly with patients' survival time (χ^2=13.181, *P*<0.001). Furthermore, subgroup analysis of each TNM stage shown only SOX9 (hi) DNMT1 (hi) expression in patients of stage III was associated with poor prognosis (**Figure 6B-D**).

Univariate and multivariate survival analysis were used to identify the independent prognostic factors. As shown in **Table 5**, univariate analysis shown histological classification, LN stage, SOX9 (hi) expression, DNMT1 (hi) expression and double (hi) expression of SOX9 and DNMT1 were prognostic factors (**Table 4**). Meanwhile, multivariate analysis shown only histological classification, LN stage, SOX9 (hi) expression, DNMT1 (hi) expression and double (hi) expression of SOX9 and DNMT1 were independent prognostic factors (**Figure 7**; **Tables 5**, **6**).

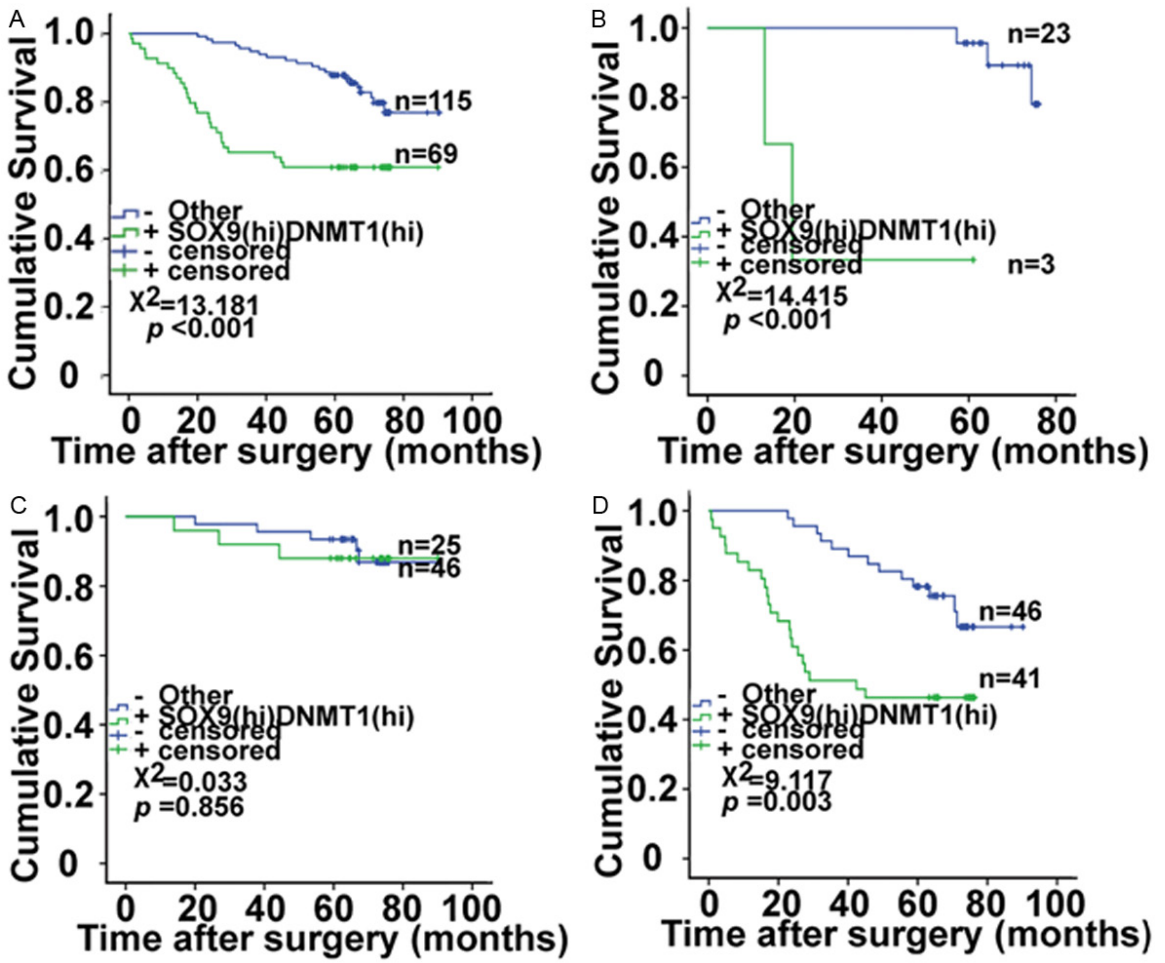

Figure 6. Survival analysis of patients with CRC according to SOX9 and DNMT1 expression. K-M curves for overall survival from 184 CRC patients (A), 36 stage I patients (B), 71 stage II patients (C), 87 stage III patients (D) according to DNMT1 expression in tumor tissues. Log-rank regression was used to test the significance.

Discussion

Tumor bulk consists of a spectrum of tumor cells, ranging from a fully differentiated epithelial state to a dedifferentiated mesenchymal state [4]. Different state of tumor cells associated with distinct functional traits. Mesenchymal cells are integral in stem cell behavior, and contribute pathologically to cancer progression [4]. During EMT, the gene expression has reprogrammed are initiated and controlled by signalling pathways that respond to extracellular cues. Among these, transforming growth factor-β (TGFβ) family signalling has a predominant role [4]. Both SOX9 and DNMT1 are downstream genes of TGFβ, and play important roles in stem cell biology, which are essential for EMT [18-20]. In clinical settings, patients with EMT marks overexpression often associate with poor prognosis [3]. In this study, we evaluated SOX9 and DNMT1 as potential biomarkers in CRC.

SOX9 is a member of a highly conserved family of transcription factors defined by their similarity to the high mobility group DNA-binding (HMG) domain of SRY, which binds to DNA and inserts into the minor curve of DNA, regulating gene transcription [5]. SOX9 plays important roles in multiple organs development and differentiation during embryogenesis, including testis, pancreas, intestine, brain, kidney, heart valves and derivatives of the neural crest, which maintains stem cell properties, restricts cellular lineage, and controls terminal differentiation, through precise regulation that differ between particular cell types and tissues [5]. For instance, it is shown by lineage tracing that

Table 5. Univariate analysis of factors associated with overall survival

Characteristics	B	SE	Wald	df	p	Exp (B) (95.0% CI)
Age	0.300	0.291	1.063	1	0.302	1.350 (0.763, 2.388)
Gender	-0.080	0.290	0.077	1	0.782	0.923 (0.523, 1.629)
Location	-0.376	0.291	1.666	1	0.197	0.687 (0.388, 1.215)
T stage	0.272	0.200	1.843	1	0.175	1.313 (0.886, 1.945)
N stage	0.814	0.179	20.611	1	0.000	2.258 (1.588, 3.209)
TNM stage	0.864	0.256	11.401	1	0.001	2.373 (1.437, 3.920)
Differentiation	0.763	0.272	7.890	1	0.005	2.145 (1.259, 3.652)
SOX9 (hi)	1.272	0.334	14.518	1	0.000	3.567 (1.854, 6.860)
DNMT1 (hi)	1.503	0.356	17.855	1	0.000	4.497 (2.239, 9.030)
SOX9 (hi) DNMT1 (hi)	1.015	0.292	12.119	1	0.000	2.760 (1.558, 4.888)

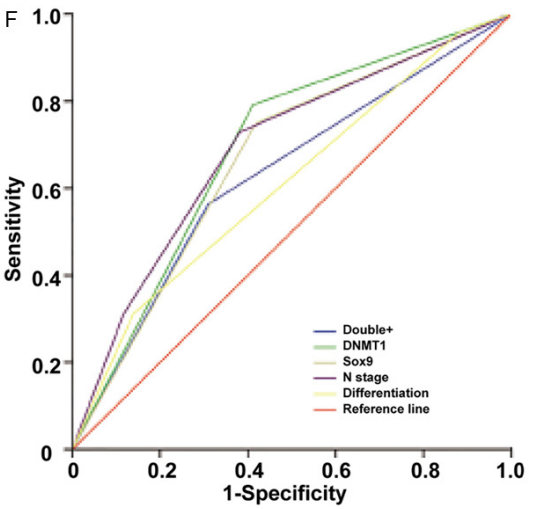

Figure 7. Variables showing significance in the multivariate analysis (**Table 7**) and the combination of SOX9 and DNMT1 levels were adopted in ROC curve analysis. Larger areas under curve indicate more predictive power of the variable.

SOX9 is expressed throughout the biliary and pancreatic ductal epithelia, which are connected to the intestinal stem-cell zone [21]. In addition, SOX9 maintains tissue homeostasis after damaged, such as in intestine, liver and pancreas [21]. Once intestine damage occurred, SOX9 expressing cells will activate and proliferate for crypt regeneration [22]. Because of its disparate functions, dysregulation of SOX9 can cause diseases, such as XX male sex reversal, fibrosis-related disorders and cancer [5]. In intestine, SOX9 is an important downstream target of WNT pathway, which is activated in colorectal cancer by mutations of APC, CTNNB, and et al [5, 23]. Gain of SOX9 copy number is detected in some primary colorectal cancers [9]. All of these may contribute to SOX9 overexpression in colorectal cancer. In intestine, not only does SOX9 repress the CDX2 and MUC2 genes, which are mature intestine marks, but also activates BMI gene expression, which represses the tumor suppressor Ink4a/Arf locus, promotes proliferation and bypasses senescence [9, 24]. All of these shows SOX9 in colorectal tumorigenesis as an oncogene. Lü B, et al not only shown SOX9 was overexpressed in colorectal cancer, but also shown overexpression of SOX9 predicted poor prognosis [12]. Furthermore, Candy PA founded overexpression of SOX9 in colorectal cancer correlated with markedly poorer survival only in 5-FU treated patients, but had no predictive effect in untreated patients [25]. And our study also shown SOX9 overexpression in CRC positively associated with histological classification of CRC patients and poor prognosis of CRC patients having received FOLFOX regimens, which is in line with Lü B's study. Recently, we have known only CRC patients of stage III benefits from chemotherapy [1]. Further analysis of each TNM stage shown SOX9 predicted poor prognosis in stage III patients, which means SOX9 may participate chemoresistance of CRC cells. Also, this is in line with Candy PA's report [25].

DNMT1 is the major DNA methyltransferase in mammalian cells and the key enzyme for the maintenance of hemimethylated DNA during DNA replication and de novo methylation during somatic cell development and differentiation, and is essential for survival of the cells [13, 14]. Genetically deletion DNMT3b in colon cancer cells only reduced global DNA methylation by less than 3%, while deletion both DNMT1 and DNMT3b reduced genomic DNA methylation by greater than 95% [14]. Further study using antisense of DNMT1, DNMT3a and DNMT3b shows DNMT1 is necessary and sufficient to maintain global methylation and aberrant CpG island methylation in human cancer cells [14]. While conditional knockout DNMT1 causes severe mitotic defects and undergoes cell death either

Table 6. Multivariate analysis of factors associated with overall survival

Characteristics	B	SE	Wald	df	*P*	Exp (B) (95.0% CI)
Age	0.299	0.317	0.890	1	0.345	1.348 (0.725, 2.507)
Gender	0.327	0.306	1.143	1	0.285	1.387 (0.761, 2.526)
Location	-0.378	0.315	1.439	1	0.230	0.685 (0.369, 1.271)
T stage	0.038	0.215	0.032	1	0.859	1.039 (0.682, 1.583)
N stage	0.779	0.357	4.761	1	0.029	2.180 (1.083, 4.390)
TNM stage	-0.198	0.454	0.190	1	0.663	0.820 (0.337, 1.997)
Differentiation	0.310	0.261	1.406	1	0.236	1.363 (0.817, 2.276)
SOX9 (hi)	3.001	1.080	7.727	1	0.005	20.115 (2.423, 166.960)
DNMT1 (hi)	2.786	1.075	6.720	1	0.010	16.214 (1.973, 133.253)
SOX9 (hi) DNMT1 (hi)	2.539	1.180	4.631	1	0.031	12.664 (1.254, 127.868)

Table 7. Prognostic values of variables for overall survival

Characteristics	Area	95% CI	*P*
N stage	0.690	0.602-0.778	0.000
Differentiation	0.609	0.515-0.703	0.025
Sox9	0.665	0.578-0.753	0.001
DNMT1	0.690	0.606-0.774	0.000
SOX9 (hi) DNMT1 (hi)	0.627	0.533-0.720	0.009

during mitosis or after arresting in a tetraploid G1 state [13]. DNMT1 overexpression are observed in many malignancies, including colon cancer, and overexpression of DNMT1 in colorectal cancer are associated with the malignant phenotype, such as poor differentiation [16]. In this study, our results also shown DNMT1 overexpression correlated with malignant phenotype, including lymph node metastasis, poor differentiation and advanced stage, especially poor prognosis, which are in line with previous report [16]. Further analysis of each TNM stage shown DNMT1 predicted poor prognosis in stage III patients, which means DNMT1 may participate chemoresistance of CRC cells.

In this study, we also found SOX9 expression positively linked to DNMT1 expression in CRC. And our results also shown SOX9 (hi) DNMT1 (hi) positively associated with malignant characteristics, such as histological classification, LN stages and TNM stages, and associated with a poor prognosis. Especially, only SOX9 (hi) DNMT1 (hi) expression in patients of stage III was associated with poor prognosis. All of these may indicate both SOX9 and DNMT1 play important roles in chemoresistance of CRC cells. Although both SOX9 and DNMT1 play important roles in development, tumorigenesis and chemoresistance, knowledge about regulation between SOX9 and DNMT1 is little. However, DNMT1 plays similar roles in intestinal and Paneth cell development [26]. Like SOX9, DNMT1 expresses on stem cell zone of intestine [26]. And similar to knockout of SOX9 in intestine, conditional gene ablation of DNMT1 in intestine causes intestinal crypt expansion in vivo and development defects [26]. All of these indicate that SOX9 may link to DNMT1 in some manner, which need to be deeply explored further and may provide new therapeutic targets for CRC treatment.

In summary, our results confirmed that EMT-related genes SOX9 and DNMT1 upregulated in CRC. Elevated expression of SOX9 and DNMT1 positively correlated with malignant characteristics of CRC, such as low differentiation, LN metastasis, advanced TNM stage and poor prognosis of patients who have received FOLFOX regimens. It was the first time to show SOX9 and DNMT1 expression positively correlated with each other and both SOX9- and DNMT1-positive expressions predicted much poorer prognosis. It can also be used as an adjunct to the UICC stage system to improve prognostication for an individual CRC patient. Exploring regulation mechanism between SOX9 and DNMT1 may provide new therapeutic targets for CRC treatment.

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Disclosure of conflict of interest

None.

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