Ioanna Balgkouranidou, Anastasios Karayiannakis, Dimitrios Matthaios, Heleni Bolanaki, Gregorios Tripsianis, Antonios Apostolos Tentes, Evi Lianidou, Ekaterini Chatzaki, Aliki Fiska, Maria Lambropoulou, George Kolios and Stylianos Kakolyris*

Assessment of *SOX17* DNA methylation in cell free DNA from patients with operable gastric cancer. Association with prognostic variables and survival

Abstract

Background: DNA methylation represents one of the most common epigenetic changes in human cancer providing important information regarding carcinogenesis. A possible role as a prognostic indicator has also been proposed. The aim of our study was to evaluate the prognostic significance of *SOX17* promoter methylation status in patients with operable gastric cancer.

Methods: Using methylation-specific PCR (MSP) we examined the incidence and prognostic significance of *SOX17* methylation status in cell free circulating DNA in the serum of 73 patients with operable gastric cancer. Fifty-one patients were male (69.9%), their median age was 65 years, 43 patients (58.9%) had regional lymph node involvement and all had a Performance Status (WHO) of 0–1.

Results: *SOX17* promoter was found to be methylated in 43 out of 73 gastric cancer serum samples examined (58.9%). All 20 control serum samples from healthy individuals were negative. Overall survival (OS) was found to be significantly associated with *SOX17* methylation (p=0.049). A significant correlation between methylation status and differentiation (p=0.031) was also observed. No other significant associations between different tumor parameters examined and *SOX17* methylation status were observed.

Conclusions: *SOX17* promoter methylation in cell free DNA of patients with operable gastric cancer is a frequent event and may provide important information regarding prognosis in this group of patients.

Keywords: cell free DNA; DNA methylation; gastric cancer; prognosis; *SOX17*.

***Corresponding author: Professor Stylianos Kakolyris,** University General Hospital of Alexandropolis, Dragana 68100, Thrace, Greece, Phone: +30 25510 74053, Fax: +30 25510 74052, E-mail: skakolyr@med.duth.gr

Ioanna Balgkouranidou, Dimitrios Matthaios and Stylianos Kakolyris: Department of Medical Oncology, Medical School, Democritus University of Thrace, Greece

Anastasios Karayiannakis and Heleni Bolanaki: Department of 2nd Surgery, Medical School, Democritus University of Thrace, Greece

Gregorios Tripsianis: Laboratory of Statistics, Medical School, Democritus University of Thrace, Greece

Antonios Apostolos Tentes: Department of Surgery, General Hospital of Didimotycho, Greece

Evi Lianidou: Laboratory of Analytical Chemistry, Department of Chemistry, University of Athens, Greece

Ekaterini Chatzaki and George Kolios: Department of Pharmacology, Medical School, Democritus University of Thrace, Greece

Aliki Fiska: Department of Anatomy, Medical School, Democritus University of Thrace, Greece

Maria Lambropoulou: Department of Histology and Embryology, Medical School, Democritus University of Thrace, Greece

Introduction

Gastric cancer is the fourth most common cancer and the second leading cause of cancer-related death worldwide [1]. Therefore, it continues to be a global healthcare problem. Over the last few decades, advances in the field of endoscopy have substantially improved early diagnosis rates of gastrointestinal malignancies [2]. Consequently, survival rates have been improved quite significantly. In this concept, identification of novel reliable biomarkers that may further improve early diagnosis or survival and predict responsiveness to specific therapies is under urgent investigation. The study of epigenetic changes in gastric cancer has the potential to improve our understanding of its etiology and provides a possibility to detect novel biomarkers that may be useful at different stages of disease.

Epigenetic processes control the packaging and function of the human genome and contribute to normal development and disease [3]. DNA methylation is one of the most frequent epigenetic events taking place in mammalian genome [4] and alterations in DNA methylation are very common in cancer cells. It is well established that methylation of normally unmethylated CpG islands correlates with the loss of expression of tumor suppressor genes in cancer

cell lines and primary tumors, including gastric cancer [5]. Methylation of these genes appears to be an early event that plays a fundamental role in the development and progression of cancer [6–8]. Detection of changes in methylation patterns as a consequence of disease or as a treatment consequence is under evaluation in the clinic and has gained increased interest as a diagnostic or prognostic indicator [9, 10]. Accordingly, an emerging catalog of specific tumor suppressor genes inactivated by DNA methylation in gastric tumors has been established [11]. Among the first documented epigenetic alterations in gastric cancer was the promoter hypermethylation of the DNA mismatch repair genes (*h MSH2*, *h MLH1*) [12].

DNA methylation can be detected in various body fluids such as blood, plasma, serum, peritoneal fluid [13], sputum [14], urine [15], gastric lavage [16], etc. In particular, cell free circulating DNA is a very promising tumor biomarker. Methylation in cell free DNA can usually be detected if the primary tumor is methylated, and it is well known that DNA fragments are frequently and abundantly found in the plasma or serum of cancer patients. These DNA fragments show the same characteristics of the primary tumor DNA [17]. A number of studies have evaluated the potential of circulating tumor-related methylated DNA in serum for the molecular diagnosis and prognosis of various cancer types [18, 19].

SOX17, a member of the Sox (Sry-related high mobility group box) family of transcription factors, is conserved in many species and participates in a variety of biological activities and developmental processes [20]. A number of Sox genes have been demonstrated to be amplified or upregulated in different tumor samples and cell lines which is indicative of an important role in human tumorigenesis. *SOX17* gene has been reported to be involved in oligodendrocyte development [21], vascular development [22] and formation of definitive endoderm [23]. Furthermore, *SOX17* has an important effect on the regulation of stem cell function, and can act as a negative regulator of b-catenin/TCF transcription activity in the Wnt signal transduction pathway [24]. Although several studies have shown that *SOX17* might also play a role in human carcinogenesis, its potential role in gastric cancer has not been fully clarified. *SOX17* is induced by Wnt activation in the early stages of gastrointestinal tumorigenesis but is downregulated by methylation during malignant progression [25, 26]. It seems that *SOX17* has a protective role at the early stages of tumorigenesis [25, 26]. Conversely, downregulation of *SOX17* contributes to malignant progression through promotion of Wnt activity.

The aim of the present study was to investigate for the first time the incidence of *SOX17* methylation pattern in cell free circulating DNA in the serum of patients with operable gastric cancer and to evaluate its possible prognostic significance.

Materials and methods

Study design

The study material consisted of 73 blood samples obtained from gastric cancer patients who underwent curative surgery with a known clinical outcome and a long follow-up period (median 56 months, range 20–111). Patient's mean age±SD was 67.07±11.09 years (range, 28–82; median age, 70.50 years) and they all had a performance status (WHO scale) of 0–1. All patients provided informed consent. Additionally, 20 blood samples obtained from healthy individuals were used as a control group. All control samples were taken from healthy friends and non-blood-related family members of patients treated in the Department of Medical Oncology. The majority of them were men, all age-matched with our patient population and received no medical care at the time of the sample collection. Their mean age±SD was 64.75±11.15 years (range, 33–77; median age, 67 years).

Sample collection and isolation of cell free DNA

Whole blood was drawn from patients pre-operatively. Blood was collected in serum clot activator tubes. Serum was obtained immediately through centrifugation at 3000 rpm for 10 min and stored at −80°C until DNA extraction. Cell free DNA from serum samples was isolated using the High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Germany). 200 μL of serum were mixed with 200 μL of working solution and 50 μL proteinase K (18 mg/mL) and incubated for 10 min at 72°C; the DNA isolation was then processed as described in the manufacturer's protocol. DNA concentration was determined in the Nanodrop ND-100 spectrophotometer (Nanodrop Technologies, USA). Carcinoembryonic antigen (CEA) levels were measured using the CEA Elecys kit (Roche Diagnostics).

Sodium bisulfite conversion

One microgram of extracted DNA was modified with sodium bisulfite, in order to convert all unmethylated, but not methylated-cytosines to uracil. Bisulfite conversion was carried out in 1 μg of extracted DNA using the EZ DNA Methylation Gold Kit (ZYMO Research Co., Orange, CA, USA), according to the manufacturer's instructions. The converted DNA was stored at −80°C until used.

Methylation-specific PCR (MSP)

The methylation status of *SOX17* gene in cell free circulating serum DNA samples was detected by methylation-specific PCR (MSP) using specific primer pairs for both the methylated and unmethyl-

ated promoter sequences. The primers amplify the region +570 +655 (GenAtlas) and are as previously described [25]. Each MSP reaction was performed in a total volume of 25 μL. One microliter of sodium bisulfite converted DNA was added into a 24 μL reaction mixture that contained 0.1 μL of Taq DNA polymerase (5 U/μL, hot start Go Taq Polymerase; Promega, USA), 5 μL of the supplied 10×PCR buffer, 2.0 μL of $MgCl_2$ (50 mmol/L), 0.5 μL of dNTP's (10 mmol/L; Fermentas) and 1 μL of the corresponding forward and reverse primers (10 μmol/L); finally dH_2O was added to a final volume of 25 μL. Sodium bisulfite treated DNA was amplified in two separate MSP reactions, one with a set of primers specific for methylated and one for unmethylated *SOX17* promoter sequences. Human placental genomic DNA (gDNA; Sigma Aldrich) methylated in vitro with SssI methylase (NEB, Ipswich, MA, USA) was used, after sodium bisulfite conversion, as fully methylated (100%) MSP positive control. The same unmethylated placental gDNA, was used, after sodium bisulfite conversion, as a negative MSP control. Thermocycling conditions used were as follows: one cycle at 95°C for 5 min, followed by 40 cycles of 95°C for 30 s, 63°C for 60 s and 72°C for 60 s, with a final extension cycle of 72°C for 4 min. MSP products for methylated and unmethylated *SOX17* promoter were fractionated on 2% agarose gels containing 40 mM Tris-acetate/1.0 mM EDTA (pH=8) and visualized by ethidium bromide staining (Figure 1).

Statistical analysis

Correlations between methylation status and clinicopathological features of the patients were assessed by the χ^2-test. Overall survival (OS) curves were calculated by using the Kaplan-Meier method and comparisons were performed using the log-rank test. p-Values <0.05 were considered statistically significant. Statistical analysis was performed by using the SPSS Windows version 17.0 (SPSS Inc., Chicago, IL, USA). The predictive power of the methylation status was tested using receiver operating characteristic (ROC) curve analysis.

Results

The methylation status of *SOX17* was evaluated in serum cell free circulating DNA samples from 73 patients diagnosed with gastric cancer in double-blinded experiments. Patient's clinicopathological characteristics (Table 1) and clinical outcome data became available after the completion of the study for the statistical correlations. Node positive patients received adjuvant combination chemotherapy consisting of docetaxel and oxaliplatin, for 4 months (6 cycles) followed by radiotherapy.

SOX17 promoter methylation status and tumor parameters

SOX17 promoter was found to be methylated in 43 out of 73 gastric cancer samples examined (58.9%) but in none of the control samples. χ^2 analysis revealed that the methylated *SOX17* promoter status was significantly more frequent in tumors with median or poor differentiation than in tumors with well differentiation (71.4% vs. 36.4%, p=0.031; odds ratio (OR)=4.4; 95% CI 1.1–17.7). There was no statistically significant association between *SOX17* promoter methylation and gender (p=0.290), age (p=0.125), site of the primary tumor (p=0.733), nodal status (p=0.723) and CEA levels (p=0.482). When age was considered as a continuous parameter, no statistically significant difference in age between patients with a methylated and patients with a non-methylated promoter (66.36±12.11 vs. 68.07±9.58, p=0.523) was found. Statistical correlations between different tumor variables and *SOX17* methylation status are given in Table 1.

SOX17 promoter methylation status and survival

After a median follow-up period of 56 months (range, 20–111 months), 38 (52.1%) patients have died as a consequence of disease progression. *SOX17* methylation was detected in 26 of these patients (26/38, 68.4%). There was

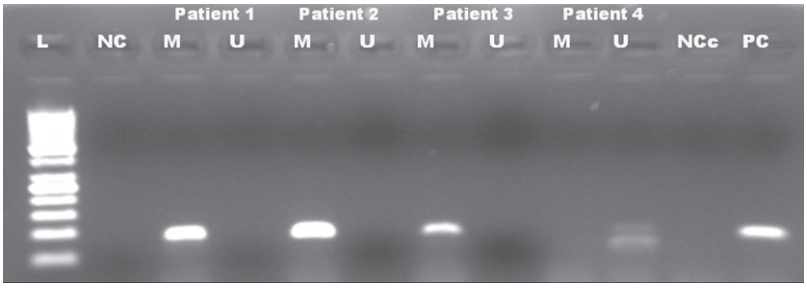

Figure 1 Agarose gel electrophoresis of methylation specific PCR products for *SOX17* methylated and unmethylated promoter, in cell free DNA of gastric cancer patient.
L, DNA ladder 50 bp; M, methylated sequence; NC, negative control; NCc, negative control conversion; PC, positive control; U, unmethylated sequence; 'converted' controls are for the MSP reaction detecting the methylated sequence.

Table 1 Clinicopathological characteristics of the patients and correlations with *SOX17* methylation status.

Patient characteristics		*SOX17*	p-Value
	n	Methylation, %	
Age			0.125
>60	56	30 (75.0)	
≤60	16	12 (53.6)	
Gender			0.290
Male	51	28 (54.9)	
Female	22	15 (68.2)	
Tumor site			0.733
Body	33	19 (57.6)	
Antrum	39	24 (61.5)	
Differentiation			0.031
Well	11	4 (36.4)	
Median-Poor	42	30 (71.4)	
Regional lymph nodes			0.723
N+	43	28 (65.1)	
N-	15	9 (60.0)	
CEA levels			0.482
<5 ng/mL	37	24 (64.9)	
>5 ng/mL	25	14 (56.0)	

a tendency towards higher incidence of death in patients with a methylated than in patients with an unmethylated *SOX17* promoter status [60.5% vs. 40.0%, p=0.081; Hazard ratio (HR)=2.0, 95% CI 1.0–3.9].

Among the entire cohort, the mean survival time±SD was 54.0±5.7 months (95% CI 42.9–65.1 months; median, 36.0 months). The mean survival time±SD of patients with an unmethylated *SOX17* promoter status was 66.9±8.6 months (95% CI 50.0–83.8) which was substantially longer to the mean survival±SD of 37.7±5.5 months (95% CI 26.9–48.6; median survival, 20 months) observed in those with a methylated *SOX17* promoter status. It should be noted that in patients with unmethylated *SOX17* promoter status the median survival time was not reached since <50% of these patients died during follow-up period.

Similarly, the Kaplan-Meier estimates of survival rates, significantly favored patients with a non-methylated *SOX17* promoter status (Figure 2, p=0.049). Further investigation with multivariate Cox proportional hazards regression analysis revealed that the methylated *SOX17* promoter status (HR=3.0, 95% CI 1.2–7.8, p=0.021) and lymph node positivity (HR=2.9, 95% CI 1.0–8.9, p=0.05) were the only statistically significant independent determinants for poor survival. Other tumor parameters such as gender (p=0.321), tumor site (p=0.110), differentiation (p=0.832), CEA levels (p=0.646) and age (p=0.149) were not statistically significant determinants of survival when they were considered either as categorical or continuous parameters.

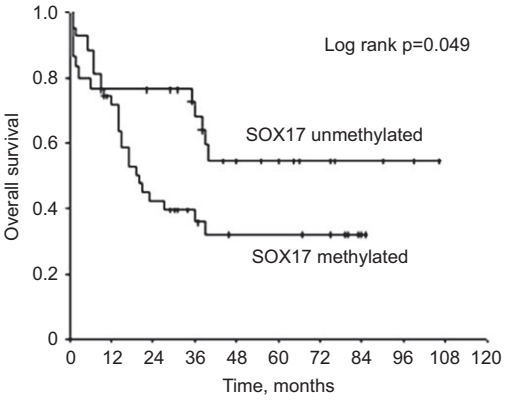

Figure 2 Kaplan-Meier estimate of overall survival (OS) for patients with early operable gastric cancer with or without *SOX17* promoter methylation (p=0.049).

ROC curve analysis showed only low predictive power for methylation status regarding survival [area under receiver operating curve (AUC) 0,600, 95% CI 0.487–0.712]. The use of methylation status for the discrimination between good and poor survival yielded moderate sensitivity of 68% (95% CI 51%–82%) and specificity of 51% (95% CI 34%–68%), with positive-predictive value of 61% and negative-predictive value of 60%.

Discussion

Epigenetic silencing due to DNA hypermethylation often leads to inactivation of the wild-type allele at sites of LOH (loss of heterozygosity) that introduces one hit in the well-known Knudson's model for tumorigenesis which accounts for loss-of-function of tumor suppressor genes [27]. DNA methylation represents a novel and very promising area of intense investigation. A number of crucial tumor suppressor genes, silenced through DNA methylation, have been evaluated in many types of cancer for their prognostic and predictive significance [9, 10]. In recent years the necessity of finding new biomarkers for early detection and prognosis in cancer, has been underlined. Cell free DNA is an optimal candidate, because it has the ability to maintain the same characteristics of the primary tumor DNA, such as oncogene expression, tumor-suppressor gene mutations and epigenetic alterations [17].

Although multiple epigenetic and genetic changes have been associated with gastric cancer, the underlying molecular mechanisms of gastric cancer pathogenesis remain unknown. A better understanding of the pathogenesis of gastric cancer may allow early detection and development of novel therapies. *SOX17* gene is a tumor suppressor gene that contributes to the Wnt/β catenin

signaling pathway. The canonical Wnt signaling plays a key role in the maintenance of integrity of intestinal stem cells and progenitor cells [28, 29]. Moreover, the constitutive activation of Wnt/βcatenin signaling causes gastrointestinal tumorigenesis [30, 31] and its promotion is important for malignant progression [32]. It has been found that *SOX17* expression is dramatically suppressed in most human gastric cancer cell lines through promoter methylation, which may result in an increase of Wnt/βcatenin signaling activity, as compared to benign gastric lesions. It is possible that downregulation of *SOX17* contributes to malignant behavior through promotion of Wnt/βcatenin signaling both in the stomach and colon.

The current study, to our knowledge, is the first one evaluating the methylation status of *SOX17* promoter methylation in cell free circulating DNA by using MSP, in patients with early operable gastric cancer. *SOX17* promoter was found to be methylated in 58.9% of tumor serum samples examined and in none of the examined healthy control serum samples. This high percentage of positivity is probably indicative that *SOX17* methylation is a common event in human gastric cancer playing a crucial role in tumor progression. This hypothesis is additionally supported by experiments in transgenic mice where a preventive role of *SOX17* in malignant progression was demonstrated [26]. Conversely, downregulation of *SOX17* through hypermethylation was associated with tumor progression. *SOX17* gene may acts as a self-protection system against tumor development [26]. The detected complete absence of *SOX17* promoter methylation in the non-malignant control plasma samples examined is also indicative that epigenetic silencing of *SOX17* may represent not only a frequent event in human gastric cancer but it might also act as a useful marker in distinguishing malignant from non-malignant gastric lesions.

Furthermore, it is of interest that *SOX17* promoter methylation status was significantly correlated with survival. Indeed, patients with an unmethylated *SOX17* promoter status had a significantly better OS. This finding is in keeping to the demonstrated role of *SOX17* as a potent inhibitor in the Wnt/βcatenin signaling pathway. *SOX17* suppresses tumor development through the inhibition of tumor cell proliferation rather than through the induction of apoptosis. Conversely, epigenetic silencing of a crucial tumor suppressor gene such as *SOX17* may also accelerate tumor spread through acquisition of an invasive and aggressive cellular phenotype facilitating gastric cancer progression. The observed significant association between *SOX17* methylation status and tumor differentiation in our study is also in keeping to this hypothesis.

In summary, using MSP, we demonstrated a high frequency of *SOX17* promoter hypermethylation in the serum of patients with operable gastric cancer and this was found to be associated with a poorer outcome. Although this finding is consistent with the known role of *SOX17* in the activation of the Wnt signaling pathway, however, the number of patients and controls examined in the present study is limited, and therefore, the predictive value of *SOX17* methylation status is uncertain.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

Received May 21, 2012; accepted January 10, 2013; previously published online February 13, 2013

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics 2002. CA Cancer J Clin 2005;55:74–108.
2. Hayatt BG, Paull PE, Wassef W. Gastric oncology: an update. Curr Opin Gastroenterol 2009;25:570–8.
3. Callinan PA, Feinberg AP. The emerging science of epigenomics. Hum Mol Genet 2006;15:95–101.
4. Laird PW. The power and promise of DNA methylation markers. Nat Rev Cancer 2003;3:253–66.
5. Bird A. DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6–21.
6. Kopelovich L, Crowell JA, Fay JR. The epigenome as a target for cancer chemoprevention. J Natl Cancer Inst 2003;95:1747–57.
7. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med 2003;349:2042–54.
8. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. Nat Rev Genet 2002;3:415–28.
9. Dammann R, Yang G, Pfeifer GP. Hypermethylation of the CpG island of Ras association domain family 1A (RASSF1A), a putative tumor suppressor gene from the 3p21.3 locus, occurs in a large percentage of human breast cancers. Cancer Res 2001;61:3105–9.
10. Fackler MJ, McVeigh M, Evron E, Garrett E, Mehrotra J, Polyak K, et al. DNA methylation of RASSF1A, HIN-1, RAR-beta, Cyclin D2

and Twist in in situ and invasive lobular breast carcinoma. Int J Cancer 2003;107:970–5.
11. Balassiano K, Lima S, Jenab M, Overvad K, Tjonneland A, Boutron-Riault MC. Aberrant DNA methylation of cancer associated genes in gastric cancer in the European Prospective Investigation into cancer and Nutrition (EPIC-EURGAST). Cancer Lett 2011;311:85–95.
12. Fleisher AS, Esteller M, Wang S, Tamura G, Suzuki H, Yin J, et al. Hypermethylation of the hMLH1 gene promoter in human gastric cancers with microsatellite instability. Cancer Res 1999;59:1090–5.
13. Ibanez de Caceres I, Battagli C, Esteller M, Herman JG. Tumor cell-specific BRCA1 and RASSF1A hypermethylation in serum, plasma and peritoneal fluid from ovarian cancer patients. Cancer Res 2004;64:6476–81.
14. Palmisiano WA. Predicting lung cancer by detecting aberrant promote methylation in sputum. Cancer Res 2000;60:5954–8.
15. Goessl C, Muller M, Straub B, Muller K. DNA alterations in body fluids as molecular tumor markers for urological malignancies. Eur Urol 2002;41:668–76.
16. Oishi Y, Watanabe Y, Yoshida Y, Sato Y. Hypermethylation of SOX17 gene is useful as a molecular diagnostic application in early gastric cancer. Tumour Biol 2012;33:383–93.
17. Swarup V, Rajeswari MR. Circulating (cell-free) nucleic acids – A promising non-invasive tool for early detection of several human diseases. FEBS Lett 2007;581:795–9.
18. Mori T, O'Day SJ, Umetani N. Predictive utility of circulating methylated DNA in serum of melanoma patients receiving biochemotherapy. J Clin Oncol 2005;23:9351–8.
19. Goebel G, Zitt M, Muller HM. Circulating nucleic acids in plasma or serum (CNAPS) as prognostic and predictive markers in patients with solid neoplasias. Dis Markers 2005;21:105–20.
20. Fu DY, Wang ZM, Chen L, Wang BL, Shen ZZ, Huang W, et al. SOX17, the canonical Wnt antagonist, is epigenetically inactivated by promoter methylation in human breast cancer. Breast Cancer Res Tr 2010;119:601–12.
21. Sohn J, Natale J, Chew LJ, Belachew S, Cheng Y, Aguirre A, et al. Identification of SOX17 as a transcription factor that regulates oligodendrocyte development. J Neurosci 2006;26:9722–35.
22. Matsui T, Kanai-Azuma M, Hara K, Matoba S, Hiramatsu R, Kawakami H, et al. Redundant roles of Sox17 and Sox18 in postnatal angiogenesis in mice. J Cell Sci 2006;119:3513–26.
23. Park KS, Wells JM, Zorn AM, Wert SE, Whitsett JA. Sox17 influences the differentiation of respiratory epithelial cells. Dev Biol 2006;294:192–202.
24. Sinner D, Kordich JJ, Spence JR, Opoca R, Rankin S, Lin SC, et al. Sox17 and Sox4 differentially regulate beta-catenin/T-cell factor activity and proliferation of colon carcinoma cells. Mol Cell Biol 2007;27:7802–15.
25. Zhang W, Glöckner SC, Guo M, Machida EO, Wang DH, Easwaran H, et al. Epigenetic inactivation of the canonical Wnt antagonist SRY-box containing gene 17 in colorectal cancer. Cancer Res 2008;68:2764–72.
26. Du YC, Oshima H, Oguma K, Kitamura T, Itadani H, Fujimura T, et al. Induction and down regulation of SOX17 and its possible roles during the course of gastrointestinal tumorigenesis. Gastroenterology 2009;137:1346–57.
27. Knudson AG. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci USA 1971;68:820–3.
28. Korinek V, Barker N, Moerer P, van Donselaar E, Huls G, Peters PJ, et al. Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. Nat Genet 1998;19:379–83.
29. Van de Wetering M, Sancho E, Verweij C, de Lau W, Oving I, Hurlstone A, et al. The β-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. Cell 2002;111:241–50.
30. Clements WM, Wang J, Sarnaik A, Kim OJ, MacDonald J, Fenoglio-Preiser C, et al. Beta-Catenin mutation is a frequent cause of Wnt pathway activation in gastric cancer. Cancer Res 2002;62:3503–6.
31. Fodde R, Smits R, Clevers H. APC signal transduction and genetic instability in colorectal cancer. Nat Rev Cancer 2001;1:55–67.
32. Fodde R, Brabletz T. Wnt/β-Catenin signaling in cancer stemness and malignant behavior. Curr Opin Cell Biol 2007;19:150–8.