SET8 expression is associated with overall survival in gastric cancer

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ABSTRACT. SET8, a member of the SET domain-containing methyltransferase, has been implicated in various biological processes. In this study, SET8 was immunostained in 100 samples of gastric cancer tissues and semi-quantified using the HSCORE method to determine the predictive value of SET8 expression levels for gastric cancer outcome. The relationship between SET8 expression and the 5-year survival rate of gastric cancer patients was assessed. High expression of SET8 was associated with a shorter survival time in gastric cancer patients, and the level of SET8 expression was found to be an independent predictor of gastric cancer outcome (relative risk = 1.939; 95% confidence interval = 1.025-3.668; P = 0.042). Analysis of SET8 levels may help in the identification of patient subgroups that are at high risk for poor disease outcomes.

Key words: Gastric cancer; Predictor; SET8; Cancer survival

INTRODUCTION

Gastric cancer ranks second in mortality amongst all cancers worldwide (Jemal et al., 2011). More than 90% of these tumors are adenocarcinomas. The prognosis is dismal, with an average 5-year survival rate of less than 20%, mainly as a result of late diagnosis. If the tumor is detected and treated before it invades the muscular layer of the stomach, the 5-year survival rate increases to 90% (Miyahara et al., 2007). Both exogenous and endogenous factors contribute to the development and progression of gastric cancer (Ali et al., 2013). It is thought that individual differences in the pathogenesis and clinical outcome are caused by genetic variations (Tahara, 1995) such as overexpression of DNA methyltransferase (Ding et al., 2008).

SET8 (also known as PR-SET7/SETD8/KMT5A), is a protein lysine methyltransferase that is present in the mitotic cell chromosome and is a member of the SET domain-containing methyltransferase family. In particular, targeting H4K20 for monomethylation (Fang et al., 2002; Nishioka et al., 2002) has been shown to have important functions in diverse biological processes, such as gene transcriptional control, replication origin modulation, genome integrity maintenance, cell cycle progression, and development (Abbas et al., 2010; Centore et al., 2010; Oda et al., 2010; Jørgensen et al., 2011; Li et al., 2011). Previous studies found that modulation of SET8 protein expression contributes to breast cancer and ovarian cancer susceptibility and affects the clinical outcome of hepatocellular carcinoma and non-small cell lung cancer (Song et al., 2009; Guo et al., 2012; Wang et al., 2012; Xu et al., 2013). Lower SET8 levels were found to be associated with longer survival in hepatic carcinoma and non-small cell lung cancer patients (Guo et al., 2012; Xu et al., 2013). In this study, we assessed the predictive capacity of SET8 on gastric cancer prognosis. We examined the correlation between SET8 expression and the clinical characteristics of gastric cancer.

MATERIAL AND METHODS

Tissue collection

We obtained gastric cancer tissues that were histologically confirmed according to the guidelines of the human tissue research committee at the hospital from 100 gastric cancer patients who underwent gastric cancer resection between 2007 and 2008 at the Thoracic Surgery Department in the Medical University. Tissues were fixed in 10% formalin immediately after resection, dehydrated in absolute ethanol, and embedded in paraffin; 5-μm thick serial sections were obtained and prepared for immunohistochemical analysis. All patients provided written informed consent for the collection of samples and subsequent analysis.

Measurement of SET8 levels in gastric cancer tissue

SET8 levels were measured using immunohistochemical staining. The tissue sections were incubated with an anti-SET8 antibody (Abcam, Cambridge, UK) at a dilution of 1:100 overnight at 4°C followed by incubation with a biotinylated secondary anti-mouse IgG antibody for 1 h at room temperature. The sections were subsequently incubated with horseradish peroxidase-conjugated streptavidin and developed using 3,3'-diaminobenzidine.

Two investigators who were blinded to the survival data scored the stained slides. The immunostaining results for all receptors were semi-quantified using the HSCORE method as

described previously (Merritt et al., 2008; Guo et al., 2012). Briefly, we calculated the score based on the estimates of the percentages of positively stained gastric cancer cells in each of 5 intensity categories (0, 1+, 2+, 3+, and 4+). The HSCORE represents the sum of each of the percentages multiplied by the weighted intensity of staining as follows: HSCORE = (i + 1)π, where i = 1, 2, 3, and 4 and π varies from 0 to 100%. A score of greater than 100% was defined as high expression, while 100% or less was defined as low expression (Figure 1).

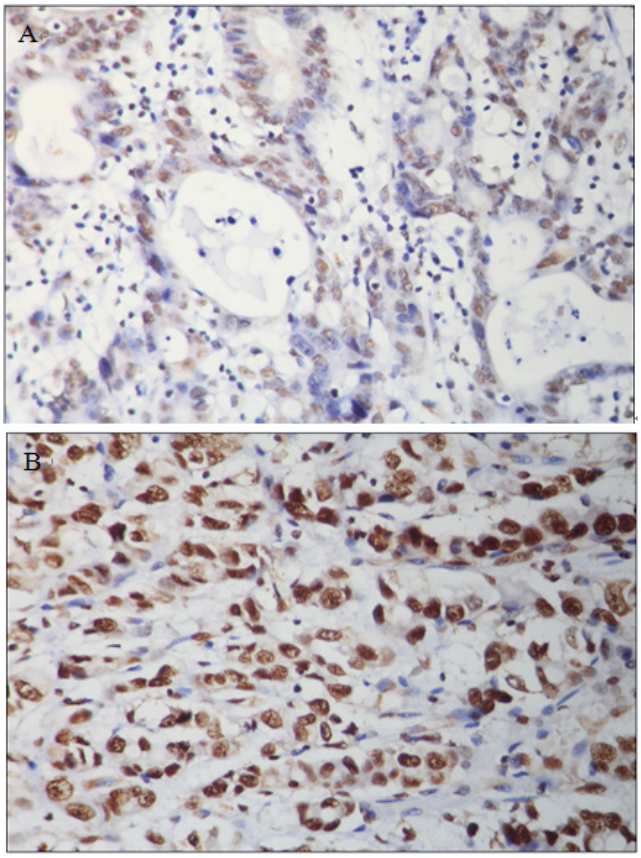

Figure 1. SET8 immunostaining in gastric cancer tissues with low expression (**A**) and high expression (**B**). Cells with a brown-stained nucleus were regarded as positive. Original magnification: 40X.

Statistical analysis

Survival data were analyzed using the Kaplan-Meier method, differences were determined using the log-rank test, and the prognostic significance of clinical characteristics was assessed using the multivariate Cox proportional hazard model. The level of statistical significance was set at $P \leq 0.05$, and all calculations were performed using the SPSS 18.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 100 gastric cancer patients, that is, 83 patients who underwent radical surgery and 17 who underwent palliative surgery, were enrolled in our study. A review of the patients was performed every 3 months for 5 years. No patients were lost during follow-up. The correlation between the data collected during the 5-year follow-up and patient clinical characteristics was analyzed using the Kaplan-Meier method and the log-rank test. Gender, age, and adjuvant chemotherapy were not associated with overall survival of gastric cancer patients; however, tumor size, operation method, and TNM classification showed an association with the 5-year survival rate at statistically significant levels (Table 1).

Table 1. Univariate analysis of clinical characteristics associated with overall survival of the gastric cancer patients.

Characteristic	Case	5-year survival rate (%)	P
Gender			
Male	73	32.9	0.296
Female	27	44.4	
Age (years)			
≤60	48	37.5	0.833
>60	52	34.6	
Size of the tumor (diameter)			
≤5 cm	29	58.6	0.002
5-10 cm	56	30.4	
>10 cm	15	13.3	
Operation method			
Radical surgery	83	43.4	0.000
Palliative surgery	17	0.0	
TNM classification			
I-II	32	65.6	0.000
III-IV	68	22.1	
Adjuvant chemotherapy			
No	48	33.3	0.455
Yes	52	38.5	
SET8			
Low expression	32	59.4	0.001
High expression	68	25.0	

The immunoreactivity of SET8 observed in gastric cancer tissues occurred mostly in the nucleus (Figure 1). Different levels of SET8 expression were observed in gastric cancer tissue, and SET8 was mostly highly expressed in gastric cancer tissues (Table 1). We graded SET8 expression in gastric cancer tissues as high or low for correlation to 5-year survival using the log-rank test. As shown in Figure 2, the Kaplan-Meier curve revealed a markedly significant distinguish between the low and high SET8 expression groups, with the lower SET8 expression group associated with a higher survival rate ($P = 0.001$).

We performed multivariate analysis using the Cox proportional hazards model for the gastric cancer-associated survival predictors described above. As shown in Table 2, the analysis revealed that SET8 expression level was an independent predictor of the survival of gastric cancer patients (relative risk = 1.939; 95% confidence interval = 1.025-3.668; $P = 0.042$). Tumor stage, operation method, and tumor size were also identified as independent predictive factors for gastric cancer outcome. These data indicate the strong predictive power of the SET8 expression on the outcome of gastric cancer patients. Univariate and multivariate analyses suggested that SET8 expression status is one of the best predictors for the outcome of gastric cancer in patients.

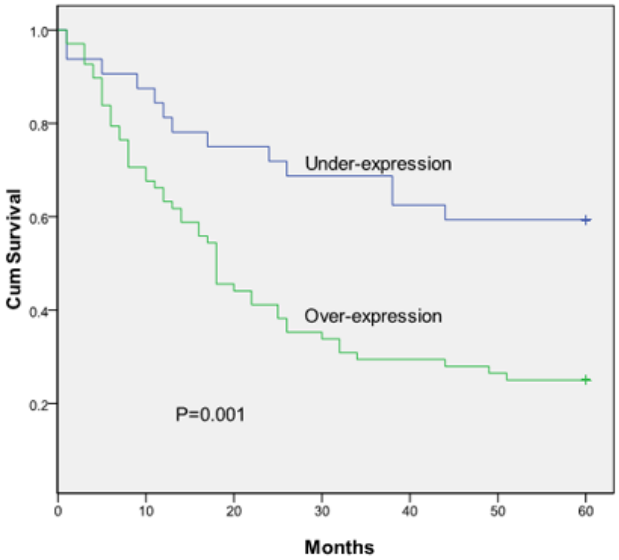

Figure 2. Inverse correlation between SET8 and the survival of gastric cancer patients. Kaplan-Meier curves depict overall survival according to the expression pattern of SET8 in patients with gastric cancer. P values were calculated using the SPSS software.

Table 2. Multivariate analysis of prognostic factors associated with overall survival in gastric cancer patients with the Cox proportional hazard model.

Factor	Relative risk	95% confidence interval	P
SET8	1.939	1.025-3.668	0.042
Size of the tumor	1.214	0.792-1.862	0.373
TNM classification	2.826	1.435-5.566	0.003
Operation method	3.618	1.937-6.755	0.000

DISCUSSION

SET8 has been implicated in multiple-biological processes and may participate in the tumor oncogenesis process. SET8 has been shown to be correlated with a series of human tumors, such as hepatocellular cancer, breast cancer, ovarian cancer, and lung cancer (Song et al., 2009; Ding et al., 2012; Guo et al., 2012; Wang et al., 2012; Xu et al., 2013). However, no studies have demonstrated a correlation between SET8 and gastric cancer. In this study, we analyzed the association between SET8 expression and gastric cancer risk and outcome, and found that the lower SET8 expression group had a higher survival rate, which is consistent with the data of a previous study (Guo et al., 2012; Xu et al., 2013).

Notably, SET8 modulates chromatin dynamics as a histone-modifying enzyme. It is well-known that epigenetic alterations in the histone code contribute to the initiation of multiple cancers (Fraga et al., 2005; Jones and Baylin, 2007). These epigenetic changes appear at an early stage of cancer development and accumulate during carcinogenic progression (Fraga et al., 2005). Thus, SET8 may be a key contributing factor to cancer development, and

downregulated SET8 may inhibit tumorigenesis and progression; low SET8 expression may modulate genome replication and stability.

Tumor protein 53 mediates the cellular response to various stresses that are mostly involved in a gene's activation or repression of the cell cycle, cell senescence, apoptosis, DNA repair, and angiogenesis (Lacroix et al., 2006). As a tumor suppressor, tumor protein 53 plays a vital role in cellular homeostasis (Papazoglu and Mills, 2007). SET8, as a methyltransferase, modulates p53 expression by specifically methylating lysine 382 histones associated with the p53 genomic sequence (Kachirskaia et al., 2007). SET8 knockdown enhances cell death and cell cycle arrest in response to DNA damage by restraining the biological function of p53 (Kachirskaia et al., 2007), and lower SET8 expression may increase the p53 level and contribute to a decreased cancer risk. Additionally, gastric cancer outcomes may be modified by altering SET8 expression, partly because of its affinity to miR-502. Previous studies have suggested that the single-nucleotide polymorphism, located within the miR-502 binding site in SET8, modulates SET8 expression and contributes to cancer risk and early cancer development (Yu et al., 2007; Song et al., 2009).

In conclusion, SET8 expression was found to be an independent prognostic marker for gastric cancer patients. Analysis of SET8 levels can help identify patient subgroups that are at a high risk for poor disease outcomes. However, the results of this study require validation in other populations and laboratory-based functional studies.

Conflicts of interest

The authors declare no conflict of interest.

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