Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

SETD2 indicates favourable prognosis in gastric cancer and suppresses cancer cell proliferation, migration, and invasion

Ziyi Chen, Chetnadevi Raghoonundun, Weidong Chen, Yanan Zhang, Wenhao Tang, Xin Fan, Xin Shi*

Department of General Surgery, Zhongda Hospital, Medical School, Southeast University, 87 Dingjiaqiao, Nanjing 210009, People's Republic of China

ARTICLE INFO

Article history:
Received 2 March 2018
Accepted 5 March 2018
Available online xxx

Keywords:
SETD2
Gastric cancer
Migration
Invasion
Biomarker

ABSTRACT

SET domain containing protein 2 (SETD2, also known as HYPB) is a 230-kD protein which is located at cytogenetic band p21.31 of chromosome 3. SETD2 is usually transformed or eradicated in multiple forms of tumours in humans. However, its primary function in gastric cancer (GC) remains unclear. In the current study, we investigated the mRNA and protein expression levels of SETD2 using immunohistochemistry, qPCR, RT-PCR, and immunoblotting. The function of SETD2 in GC cells was investigated by MTT and transwell assays. Our results revealed remarkably lower levels of SETD2 mRNA and protein in the tumour samples than in tumour-adjacent tissues. Decreased expression of SETD2 mRNA was observed in 122 (79.7%) of 153 primary tumour tissue samples. On the basis of the overall survival analysis, we could interpret that a low expression of SETD2 was correlated with a poor prognosis ($P < 0.001$, log-rank test). Multivariate survival analysis indicated that SETD2 was an obvious prognostic factor in patients with GC. SETD2 overexpression in GC cell lines significantly inhibited cell proliferation, migration, and invasion. Altogether, the investigation demonstrated the clinical significance of SETD2 expression, supporting the fundamental principle that a decrease in its level is an unfavourable event in the progression and prognosis of GC. Therefore, down-regulated SETD2 can presumably be a potential negative prognostic and progression marker for GC.

1. Introduction

SET domain containing protein 2 (SETD2, also known as HYPB) is a 230-kD protein which is located at cytogenetic band p21.31 of chromosome 3, the chromosome associated with telomerase suppression and the permanent growth arrest of tumour cells [1,2]. SETD2 trimethylates histone H3K36, which has a transcriptional activation domain and hyperphosphorylates RNA polymerase II (RNAPII). The SET domain was initially determined to be in parts of the poly comb group (PcG), trithorax group (*trxG*), and *Su(var)* genes, and were therefore named after the genes *Su(var)3–9*, enhancer of zeste (*E(z)*), and trithorax (*trx*) [3]. Most histone methyltransferases consist of functional domains, such as protein–DNA/RNA interaction domains, protein–protein interaction domains, and transcriptional activation or repression domains [4]. Histone methyltransferases are directed towards a few protein compounds and effectuate certain precise activities [5].

A previous report had shown that histone modifications play important roles in gene regulation, such as acetylation, phosphorylation, methylation, and ubiquitination [6]. These modifications generate both supporting and opposing signals that are involved in the transcriptional activity of a gene, allowing the initiation of RNA polymerase transcription by engaging some protein compounds or by bringing about some changes in the structure of chromatin [5], and serve as a marking system that plays a key role in exhibiting and maintaining gene expression programs during cellular differentiation [2]. It has been proven that SETD2 can act as a tumour suppressor gene in renal carcinoma, breast carcinoma, and early T-cell precursor acute lymphoblastic leukaemia [7–9]. Another previous study has shown that SETD2 could react with p53 and modulate its transcription factor task. The result showed that SETD2 targeted the P2 promoter of the *hdm29* gene and decreased its expression, thereby enhancing the stability of the p53 protein. These findings show that there could be discerning regulation in the transcription of the subset genes by SETD2 through its cooperation with the transcription factor p53 [10].

* Corresponding author.
E-mail address: dr_shix@126.com (X. Shi).

https://doi.org/10.1016/j.bbrc.2018.03.022
0006-291X/

The mutation or deletion of SETD2 is a frequent event in several kinds of human tumours, including clear cell renal carcinoma [11,12]. However, the clinical significance and primary function of SETD2 in GC remain unknown. The purpose of this investigation was to explore the associations of SETD2 expression with the invasion, proliferation, and migration properties of gastric cancer (GC) cells, as well as its relationship with clinicopathological parameters of this disease. To the best of our knowledge, this is the first known study to evaluate the direct association between SETD2 and GC.

2. Materials and methods

2.1. Cell culture and clinical samples

The GC cell lines AGS, HGC-27, and GES-1 were obtained from the Cancer Institute of the Chinese Academy of Medical Science. The cell lines were maintained in RPMI-1640 medium (Life Technologies Inc., USA) supplemented with 10% foetal bovine serum (FBS; Life Technologies Inc.) at 37 °C in a humidified incubator containing 5% CO_2. From March 2006 to September 2007, a total of 153 tumour specimens and paired normal tissues were collected from Zhongda Hospital affiliated to Southeast University. Patients were verified to have GC at initial diagnosis, and none of them had received chemotherapy or radiotherapy. The samples were stored in liquid nitrogen until use. This study was approved by the Medical Ethics Committee of Zhongda Hospital.

2.2. RT-PCR and quantitative PCR

TRIzol reagent (Invitrogen, USA) was used to extract total RNA from the cells and tissues. The cDNA was synthesized using a reverse transcription kit (#A3500, Promega, USA), and quantitative PCR (qPCR) was conducted using primers and the SYBR Green PCR Master Mix (#A25780, Thermo Fisher Scientific, USA). The transcript levels of genes were analysed by the $2^{-\triangle\triangle Ct}$ method. RT-PCR was conducted using primers and *Taq* DNA polymerase (#M7122, Promega). The primers for SETD2 and H3 were designed and purchased from Sangon Biotech (China).

2.3. Immunoblot assay

RIPA lysis buffer (#R0278, Sigma-Aldrich, USA) and a protease inhibitor cocktail (#P8340, Sigma-Aldrich) were used to obtain total cell lysates. The proteins were separated by 10% SDS-PAGE. Polyclonal anti-SETD2 (#sc-99451, Santa Cruz Biotechnology, USA) and anti-histone H3 (#sc-8654, Santa Cruz Biotechnology) antibodies were used to detect SETD2 and H3 protein expression, respectively. Labelling was visualised with an enhanced chemiluminescence system kit (#WBKLS0500, Millipore, USA). Histone H3 was used as the loading control.

2.4. Establishment of HGC-SE and AGS-SE clones

Approximately 1×10^5 HGC-27 or AGS cells in 10% FBS-supplemented RPMI 1640 medium were seeded into 35-mm plates and cultured overnight. The plasmid carrying SETD2 was obtained from Dr. Zhu Chen [13]. Lipofectamine 3000 (#L3000001, Thermo Fisher Scientific) was used to transfect the cancer cells with either the full-length SETD2 cDNA-carrying vector or the empty vector. The transfected cells were selected with G418 (100 mg/ml) for 14 days before separating the individual clones.

2.5. Cell proliferation assay

The MTT assay was used to evaluate cell proliferation. In brief,

Table 1
Univariate and multivariate statistics of the prognostic value of gender, age, size, lymph node metastasis, stage, and expression of SETD2 for survival (5years) in gastric cancer.

Parameters	No. (%)	High (%)	Low (%)	Univariate		Multivariate		
	N = 153	N = 31	N = 122	P-value	P-value	Risk ratio	95% CI	P-value
Gender				[b]P = 0.626	P = 0.181	1.246	0.631–2.459	P = 0.526
Male	80 (52.3)	15(18.8)	65(81.2)					
Female	73 (47.7)	16(21.9)	57(78.1)					
Age(years)				[b]P = 0.576	P = 0.426	0.958	0.489–1.878	P = 0.901
≥64[a]	82 (53.6)	18(22.0)	64(78.0)					
<64	71 (46.4)	13(18.3)	58(81.7)					
Size(cm)				[b]P < 0.001	P < 0.001	3.005	1.116–8.091	P = 0.029
<3	23(15.0)	15(65.2)	8(35.8)					
≥3	130 (85.0)	16(12.3)	114(87.7)					
TNM stage				[b]P < 0.001	P < 0.001	2.076	1.037–4.158	P = 0.039
I,II	26 (17.0)	14(53.8)	12(46.2)					
III,IV	127 (83.0)	17(13.4)	110(86.6)					
Lymph node metastasis				[b]P < 0.001	P < 0.001	5.081	1.804–3.820	P = 0.038
Negative	18 (11.8)	14(77.8)	4(22.2)					
Positive	135 (88.2)	17(12.6)	118(87.4)					
SETD2 expression					P < 0.001	1.428	0.201–0.910	P = 0.027
≥1	31 (20.3)							
<1	122 (79.7)							

Results of univariate analyses using the log-rank test and multivariate analyses using the Cox proportional hazards model of prognostic factors for overall survival. CI, confidence interval.
[a] Divided by median age of cases.
[b] Pearson's $\chi 2$ test.

Please cite this article in press as: Z. Chen, et al., SETD2 indicates favourable prognosis in gastric cancer and suppresses cancer cell proliferation, migration, and invasion, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.03.022