PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

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RAPID COMMUNICATION

Hypermethylation and aberrant expression of Wnt antagonist secreted frizzled-related protein 1 in gastric cancer

Cheng-Hai Zhao, Xian-Min Bu, Ning Zhang

Cheng-Hai Zhao, Ning Zhang, Department of Pathophysiology, School of Basic Medicine, China Medical University, Shenyang 110001, Liaoning Province, China
Xian-Min Bu, Department of General Surgery, the Second Affiliated Hospital, China Medical University, Shenyang 110004, Liaoning Province, China
Supported by Liaoning Education Divison Foundation, No. 05L557
Correspondence to: Dr. Cheng-Hai Zhao, Department of Pathophysiology, School of Basic Medicine, China Medical University, Shenyang 110001, Liaoning Province, China. zhaochenghai1@sina.com
Telephone: +86-24-23256666-5362
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Abstract

AIM: To identify the methylation of secreted frizzled-related protein 1 (SFRP1) in gastric cancer and to investigate the aberrant expression of SFRP1 and its correlation with the clinical pathological features of patients.

METHODS: We determined SFRP1 methylation and SFRP1 mRNA expression in 3 gastric cancer cell lines SGC-7901, BGC-823, HGC-27, from 52 primary gastric cancer specimens and matched tumor adjacent tissue specimens by methylation-specific (MSP) PCR and RT-PCR respectively. Fisher's exact test was used to analyze the statistical association between clinical pathological data and aberrant expression of SFRP1.

RESULTS: In 3 cancer cell lines, BGC-823 and HGC-27 had methylated SFRP1 and lost SFRP1 mRNA expression. After treatment of BGC-823 and HGC-27 with the demethylating agent, 5-aza-2'-deoxycytidine, SFRP1 was re-expressed. In 52 primary gastric cancer specimens and matched tumor adjacent tissue specimens, hypermethylation of SFRP1 was detected in 23 (44%) and 8 (15%) specimens respectively ($\chi^2 = 10.34$, $P < 0.01$). Loss of SFRP1 expression was detected in 17(33%) and 6 (12%) specimens respectively ($\chi^2 = 6.75$, $P < 0.01$). There was a significant correlation between SFRP1 hypermethylation and SFRP1 expression loss. SFRP1 expression was also correlated significantly with tumor stage and lymph node status, but not with patient sex, age and histological type.

CONCLUSION: SFRP1 inactivation is a common and early event caused mainly by hypermethylation in gastric cancer. SFRP1 expression loss may be correlated with tumor metastasis in primary gastric cancer.

Key words: Secreted frizzled-related protein 1; Wnt; Hypermethylation

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INTRODUCTION

Carcinogenesis is a complex process involving a series of genetic and epigenetic changes. Aberrant methylation is recognized as an important pathway leading to gene silencing. Hypermethylation in CpG islands and silencing of tumor-related genes in human cancer have been the focus of study in the last decade.

Secreted frizzled-related protein 1 (SFRP1) found in 1987 by several different groups[1-3], contains a cysteine-rich domain (CRD) which share 30%-50% sequence similarity with those of Wnt receptor frizzled proteins. Through the CRD, SFRP1 can antagonize Wnt signaling by interacting with Wnt ligand. As Wnt signaling pathway plays an important role both in embryo development and in proliferation, differentiation and apoptosis in adult tissues. Thus aberrant activation of Wnt pathway may induce tumorigenesis. As a Wnt inhibitor, SFRP1 down-regulation caused by hypermethylation has been found in several kinds of cancer[4-8].

In this study, we detected the methylation status and aberrant expression of SFRP1 in gastric cancer. We also analyzed the correlation between the expression of SFRP1 and clinical pathologic characteristics of primary gastric cancer.

MATERIALS AND METHODS

Cell lines, cancer and matched adjacent tissue samples

Human gastric cancer cell lines SGC-7901, BGC-823 and HGC-27 (From KUNKEN Bio-reagent Corp. Shanghai, China) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, penicillin (100 IU/mL), and streptomycin (100 μg/mL). Primary gastric cancer and

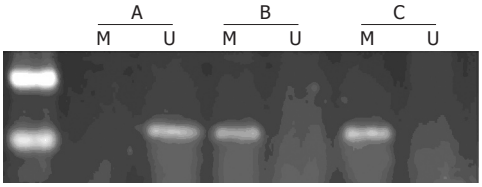

Figure 1 Hypermethylation of SFRP1 in BGC-823 (B) and HGC-27 (C) but not in SGC-7901 (A). M: methylated; U: unmethylated.

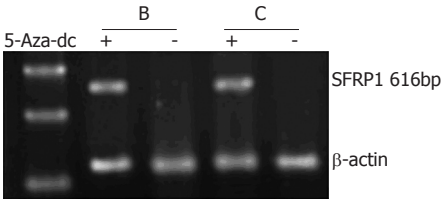

Figure 3 Expression of SFRP1 mRNA after treatment with 5-aza-2'-deoxycytidine in BGC-823 (B) and HGC-27 (C).

matched adjacent tissue samples were obtained from patients who underwent operation at the Second Affiliated Hospital, Chinese Medical University. The samples were frozen in liquid nitrogen immediately after surgery. Haematoxylin and eosin staining was used to confirm that cancer samples were consisted mostly of tumor cells and there were no tumor cell in tumor adjacent tissue samples.

DNA and RNA extraction

DNA was extracted by a standard phenol/chloroform extraction and ethanol precipitation procedure. RNA was isolated by TRIZOL (Takara Corp.) according to the protocols supplied by the manufacturer.

Semi-quantitative reverse transcription-PCR

RT-PCR was performed by *Takala* RNA PCR 3.0 Kit. cDNA was synthesized from 1 μg SFRP1 RNA using random 9 primer and AMV reverse transcriptase. Cycle condition was 1 cycle at 30℃ for 10 min, at 42℃ for 25 min, at 99℃ for 5 min and at 5℃ for 5 min. For PCR, the SFRP1 primer sequences[7] are F (5'-TCTACACCAAGC CACCTCAG-3') and R (5'-CAGTCACCCCATTCTTCA GG-3'). Cycle condition was 1 cycle at 94℃ for 2 min; 30 cycles at 94℃ for 30 s, at 60℃ for 30 s and at 72℃ for 2 min.

Methylation-specific PCR

The methylation status of SFRP1 was detected by GENMED MSP Kit (GENMED, Shanghai, China). The procedure was performed according to its protocol. The primers[7] for methylated sequence of SFRP1 are F (5' -TGTAGTTTTCGGAGTTAGTGTCGCGC-3') and R (5' -CCTACGATCGAAAACGACGCGAACG-3'), and those for unmethylated sequences are F (5'-GTTTTGTAGTTT TTGGAGTTAGTGTTGTGT-3') and R (5'-CTCAACCT ACAATCAAAAACAACACAAACA-3'). Cycle condition was 1 cycle at 95℃ for 5 min; 35 cycles at 95℃ for 30 s, at 60℃ for 30 s and at 72℃ for 30 s.

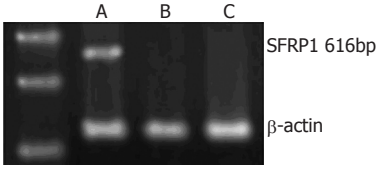

Figure 2 Expression of SFRP1 mRNA in SGC-7901 (A) but not in BGC-823 (B) and HGC-27 (C).

Table 1 Hypermethylation and expression loss of SFRP1 in primary gastric cancer and matched cancer adjacent tissue samples

	n	Hypermethylation (+)	Expression (-)
Gastric cancer	52	23	17
Adjacent tissue	52	8	6
P		$\chi^2 = 10.34$, $P < 0.01$	$\chi^2 = 6.75$, $P < 0.01$

5-aza-2'-deoxycytidine treatment

Cells were seeded at a density of 3×10^4 cells/cm^2 in a four well plate on d 0, and exposed to 5-aza-2'-deoxycytidine (Sigma, 1 μmol/L) on d 1, 2, and 3. After each treatment, the cells were cultured in fresh medium. Control cells were incubated without the addition of 5-aza-2'-deoxycytidine. Cells were harvested in d 4 for RNA extract.

Statistical analysis

Methylation and expression status of SFRP1 in primary pancreatic cancer and adjacent tissue samples were compared by χ^2 test. Fisher's exact test was used to study the statistical association between clinical pathologic data and aberrant expression of SFRP1. Differences were considered statistically significant when *P* values were less than 0.05.

RESULTS

Hypermethylation and expression of SFRP1 in gastric cancer cell lines

Hypermethylation of SFRP1 was found in BGC-823 and HGC-27 by MSP (Figure 1). Expression of SFRP1 mRNA was detected in SGC-7901, but not in BGC-823 and HGC-27 (Figure 2). After treatment of BGC-823 and HGC-27 with the demethylating agent, 5-aza-2' -deoxycytidine, SFRP1 mRNA was re-expressed in BGC-823 and HGC-27 (Figure 3).

Hypermethylation and expression of SFRP1 in primary gastric cancer and matched cancer adjacent tissue samples

In 52 primary gastric cancer samples, hypermethylation of SFRP1 was detected in 23. In matched cancer adjacent tissue samples, 8 were found with SFRP1 hypermethylation. The hypermethylation rate in primary gastric cancer samples was significantly higher than that in matched cancer adjacent tissue samples ($\chi^2 = 10.34$, $P < 0.01$). Expression of SFRP1 mRNA was not found in 17 gastric cancer samples and 6 matched cancer adjacent tissue samples respectively. The difference was also significant ($\chi^2 = 6.75$, $P < 0.01$) (Table 1).

Table 2 Correlation of SFRP1 expression with SFRP1 hypermethylation and clinical pathologic patient data

Variable	Categorisation	*n*	SFRP1 (-)	*P*
Sex	Male	34	11	0.24
	Female	18	6	
Age at diagnosis	> 50	30	12	0.10
(yr)	≤ 50	22	5	
Tumor stage1	T1 + T2	25	4	0.01
	T3 + T4	27	13	
Lymph node status	N0 + N1	36	8	0.02
	N2 + N3	16	9	
Histological type	Diffused	31	12	0.13
	Intestinal	21	5	
Methylation status	Methylated	28	13	0.02
	Unmethylated	24	4	

[1]According to UICC: TNM classification of malignant tumors.

Correlation analysis of SFRP1 expression, SFRP1 hypermethylation and clinical pathologic data in primary gastric cancer

Fisher's exact test was used to analyze the correlation between SFRP1 expression and SFRP1 methylation and other clinical pathologic patient data. A significant correlation was found ($P = 0.02$) between SFRP1 hypermethylation and SFRP1 expression loss. SFRP1 expression was also correlated significantly with tumor stage ($P = 0.01$) and lymph node status ($P = 0.02$), but not with patient sex, age, histological type (Table 2).

DISCUSSION

Hypermethylation in promoter CpG islands and diminished expression have been reported to be present in a number of tumor-related genes in gastric cancer, including *p*16[9], hMLH1[10], E-cadherin[11], APC[12], MGMT[13], RASSF1A[14], DAP-kinase[15], RUNX3[16], GSTP1[17]. In addition, many studies have shown that concurrent hypermethylation of multiple tumor-related genes in gastric cancer, is also termed CpG island methylator phenotype (CIMP). Recently, several other tumor-related genes have been reported with hypermethylation in CpG island in gastric cancer, such as CHFR[18], HLTF[19], RIZ1[20], HRK[21], SOCS-1[22], BNIP3[23]. The identification of genes targeted by hypermethylation may provide insights into the mechanisms underlying the inactivation of tumor suppressive pathways in gastric cancer.

Wnt signaling pathway plays an important role in cell proliferation, differentiation and apoptosis in adult tissues. Aberrant activation of Wnt signaling in tumorigenesis has been reported frequently. Overexpression of members of Wnt family, such as Wnt1, Wnt2 and Wnt3a, has been found in several kinds of human cancer[24-26]. Down-regulation of Wnt inhibitor DKK family has also been found in human cancer[27,28]. As another kind of Wnt inhibitor, SFRP1 may promote cell apoptosis[2]. Additionally, the SFRP1 gene has been found at 8p11.2, a site of frequent loss of heterozygosity, so it may be a putative tumor suppressor gene. SFRP1 down-regulation has also been found in several kinds of human in recent years. Most of these reports showed that SFRP1 expression loss mainly caused by promoter hypermethylation is an important epigenetic gene silencing mechanism.

In this study, we analyzed the expression status of SFRP1 in gastric cancer. Our results showed that primary gastric cancer had SFRP1 expression loss which was significantly correlated with SFRP1 hypermethylation. The hypermethylation rate in primary gastric cancer samples was significantly higher than that in tumor adjacent tissue samples, suggesting SFRP1 hypermethyaltion and subsequent expression loss occur early and play an important role in gastric cancer. At the same time, SFRP1 expression loss was significantly correlated with tumor stage and lymph node status, indicating that SFRP1 expression loss might be associated with poor prognosis in gastric cancer.

As we know, Wnt signaling is divided into canonical pathway Wnt/β-catenin and non-canonical pathway, including planar cell polarity pathway and Wnt/Ca^{2+} pathway. As we did not measure the level of β-catenin, we could not determine the pathway through which SFRP1 expression loss take part in the gastric carcinogenesis. The detailed mechanism still needs more studies. Because the epigenetic alteration usually is reversible, demethylating drugs may be used in the treatment of gastric cancer.

COMMENTS

Background

Wnt signaling pathway plays an important role in cell proliferation, differentiation and apoptosis in adult tissues. Aberrant activation of Wnt signaling in tumorigenesis has been reported frequently. As a Wnt inhibitor, SFRP1 down-regulation has been s found in several kinds of human cancer in recent years.

Innovations and breakthroughs

In this study, we analyzed the methyaltion and expression status of SFRP1 in gastric cancer. SFRP1 inactivation was found to be a common and early event caused mainly by hypermethylation in gastric cancer.

Application

Because many tumor-related genes are methyalted in gastric cancer, demethylating drugs may be used in treatment of gastric cancer.

Peer review

The authors examined SFRP1 methylation and SFRP1 mRNA expression in 3 gastric cancer cell lines, 52 primary gastric cancers and matched adjacent tissues by methylation-specific PCR and RT-PCR, respectively. Two of the 3 cancer cell lines had methylated SFRP1 and lost mSFRP1 mRNA expression. Hypermethylation of SFRP1 was detected in 23 gastric cancers and 8 adjacent tissues, respectively. There was a significant correlation between SFRP1 hypermethylation and SFRP1 expression loss. The authors concluded that SFRP1 inactivation is a common and early event which is caused mainly by hypermethylation in gastric cancer.

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