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Aberrant methylation of suppressor of cytokine signaling-1 gene in human gastric carcinomas

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ABSTRACT

Suppressor of cytokine signaling (SOCS)-1 inhibits signaling of the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathway by several cytokines and has tumor suppressor activity. Methylation of the SOCS-1 CpG island has been shown to inactivate the SOCS-1 gene in certain human cancers. In our study, we investigated methylation status of the SOCS-1 gene by methylation-specific PCR in 45 gastric carcinoma (GC) tissues, 18 corresponding nonneoplastic mucosae and 10 normal gastric mucosae from healthy young individuals. In addition, SOCS-1 mRNA levels were examined in 30 GCs by quantitative RT-PCR. Hypermethylation of the SOCS-1 gene was detected in 21 (46.7%) of 45 GC tissues and in 2 (11.1%) of 18 corresponding nonneoplastic mucosae; the incidence was significantly different ($p = 0.0081$). None of the 10 normal gastric tissues from healthy individuals showed hypermethylation. Methylation of the SOCS-1 gene was associated with lymph node metastasis, advanced tumor stage and reduced expression of SOCS-1 in GC tissues ($p = 0.0032$, 0.0040 and 0.0026, respectively). Reduced expression of SOCS-1 in GC tissues was associated with lymph node metastasis and advanced tumor stage ($p = 0.0143$ and 0.0025, respectively). Our results suggest that transcriptional inactivation of the SOCS-1 gene by hypermethylation may be involved in development, progression and metastasis of GC.

KEYWORDS

SOCS-1; Gastric cancer; Methylation.

INTRODUCTION

Gastric cancer is one of the most common gastrointestinal malignancy, is still taking an active radical surgery and adjuvant therapy, but the 5-year survival rate is still low. The mechanism is closely related to changes in gastric cancer genes, but changing the non-gene epigenetic changes are more and more attention. If abnormal methylation of oncogenes, histone acetylation and so on. In this study, methylation-specific PCR reaction of gastric SOCS-1 gene methylation status of CpG islands, explore the SOCS-1 gene abnormality in the development of the role and possible mechanisms of gastric cancer.

MATERIALS AND METHODS

1) Clinical data resented specimens taken from 20012 to 20013, Zhongnan Hospital surgical tumor resection specimens 45 cases and 18 cases of gastric cancer adjacent tissue were pathologically confirmed gastric cancer, and there were no preoperative radiotherapy, chemotherapy and immunotherapy history. Including 28 males and 17 females, with an average 52 years of age. Among them, 26 cases with lymph node metastasis; clinical stage: Ⅰ + Ⅱ stage 21, Ⅲ + Ⅳ stage 24 cases. Well-differentiated 22 cases, 33 cases of poorly differentiated. Another 10 cases of normal gastric mucosa of healthy people as controls. Prior to analysis specimens are deposited in - 80 °C.

2) Methylation-specific PCR reactions Biological Engineering Company in accordance with Hua Shunsheng DNA extraction kit operating method to extract DNA from stomach cancer specimens. After extracting DNA quantification according to[1] Herman and other methods, little improvement carried sodium bisulfite modification. Sodium bisulfite DNA using the modified methylation-specific PCR amplification reaction[2]. The reaction unmethylated primers SOCS-1 UM (unmethylation unmethylated), S : 5'-TTA TGA GTA TTTGTG TGT ATT TTT AGG TTG GTT-3', AS :5'-CAC TAA CAA CAC AAC TCC TAC AAC AACCA-3'; The methylation reaction primerSOCS-1 M(methylation), S : 5'-TTC GCG TGT ATT TTT AGGTCG GTC-3', AS : 5'-CGA CAC AAC TCC TACAAC GAC CG-3'. The PCR reaction at 95 °C hot start 15 min (HotStarTaq PCR, HotStarTaq DNA Polymerase, QIAGEN), and then 95 °C 30 s, 60 °C 45 s, 72 °C 1 min, 40 cycles of the last cycle of 72 °C for 4 min. Each 10 μl PCR reaction product was taken directly added 3% agarose gel electrophoresis, and a developing apparatus under UV.

3) SOCS1 gene expression by Real-time quantitative PCR : Using QIAGEN RNA extraction kit RNeasy Protect MidiKit, according to kit from tumor tissue, the corresponding normal tissue extracted RNA, quantified by the QIAGENSensiscript RT Kit to the reverse transcription of RNA to cDNA saved. S : 5'-AACTGC TTT TTC GCC CTT AGC-3', AS : 5'-CAGCTC GAA GAG GCA GTC G-3'; GAPDH S : 5'-GAC CAC AGT CCA TGCCAT CAC-3', AS : 5'-GTC CAC CAC CCT GTTGCT GTA-3'. The reaction system is as follows: cDNA 0.5μl, primer 0.15 μl, SYBR Green master mix 5μl (AppliedBiosystems, ABI), RNA-water 4.35μl, a total reaction volume of 10μl, ABI PRISM7900 Sequence Detection System (AppliedBiosystems, ABI) for PCR reaction was conditions were 50 °C 2 min, 95 °C 10 min, and then 2-step PCR reaction, 95 °C 15 s, 60 °C 60 s, 40 cycles, data collection and analysis by the ABI PRISM7900. GAPDH as an endogenous reference gene, in a standardized relative expression amount of each specimen SOCS-1 gene, all values relative to GAPDH gene expressed in multiples of increase or decrease. The value of each sample to gene replication cycle threshold (Ct limit cycles) expression. Number (⊿ Ct) gene expression of the target gene and the endogenous reference gene GAPDHCt said difference value, and the relative expression of the gene (⊿ ⊿ Ct) to the specimen and the reference specimens tested ⊿ Ct represents the difference, then the purpose of the relative expression of the gene to 2 - ⊿ ⊿ Ct means[3].

4) Statistical Methods SPSS1010 statistical software, the relationship between gene expression and clinicopathological features using $\chi 2$ test for paired data to $P < 0.05$ was considered statistically significant.

RESULT

Gastric cancer specimens SOCS-1 gene methylation status

45 cases of gastric cancer specimens, 21 cases have SOCS-1 gene aberrant methylation of CpG islands, and adjacent tissues, only 18 cases of methyl 2 cases of a gene of 10 cases of normal tissues and no SOCS-1 gene of performance (see Figure 1).

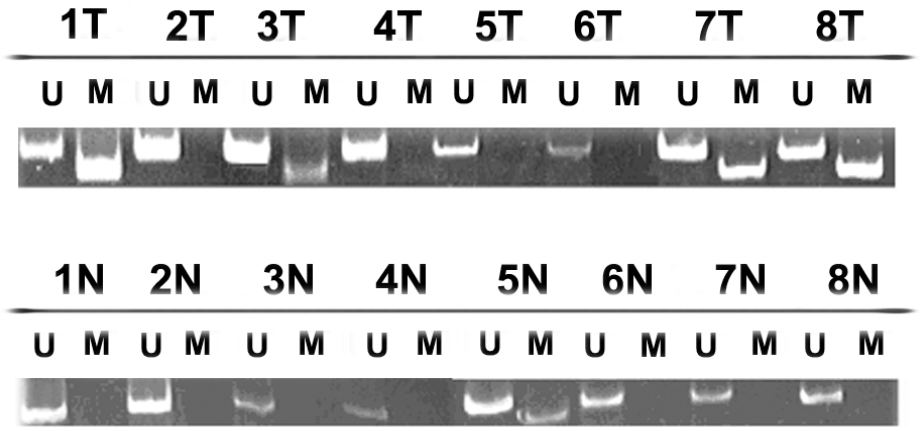

Figure 1 : Article unmethylated PCR product was 175bp bands, and U token, methylation of the PCR product was 160bp bands, and M notation; T on behalf of the tumor tissue, N for non-neoplastic mucosa adjacent organization, found that methylation of tumor samples 1,3,7,8, and adjacent tissue samples occurred only five paracancerous methylation.

Relationship SOCS-1 methylation and clinical

Aberrant gene methylation status and clinicopathological features of gastric cancer patients analyzed and found SOCS-1 gene methylation and the patient's age, sex has nothing to do with the degree of tumor differentiation, lymph node metastasis and TNM stage were correlated (see TABLE 1).

TABLE 1 : SOCS-1 methylation status with clinicopathological features of gastric cancer relationship

	***SOCS-1* methylation**		***p*-value**
	Positive	**Negative**	
Sex			
male	12	16	0.5109
female	9	8	
age			
≤65	7	9	0.7708
>65	14	15	
differentiation			
Well-differentiated	6	16	0.0108
poorly-differentiated	15	8	
Lymph node metastasis			
none	4	15	0.0032
have	17	9	
TNM stage			
Ⅰ+Ⅱ	5	16	0.0040
Ⅲ+Ⅳ	16	8	
pattern			
Intestinal	15	13	0.2334
Mixed	6	11	

SOCS-1 gene expression real-time PCR results

Methylation group SOCS-1 gene expression was significantly lower than the relative unmethylated group, there are significant differences (P = 0.0067) (Figure 2), indicating that SOCS-1 gene aberrant CpG island methylation was significantly inhibiting the expression of SOCS-1 gene.

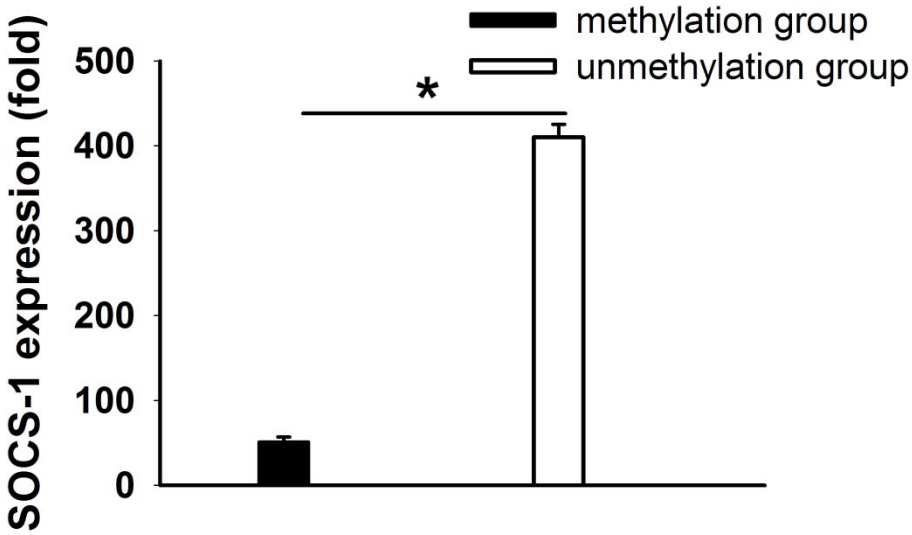

Figure 2 : Relationship between SOCS-1 expression and DNA methylation, ＊P<0.01

DISCUSS

In our study, we found that gastric cancer SOCS-1 gene in DNA methylation and SOCS-1 gene DNA hypermethylation and its mRNA expression is related, indicating that SOCS-1 gene hypermethylation SOCS in gastric cancer -1 transcriptional inactivation plays an important role. SOCS-1 methylation were significantly different in adjacent tissues and gastric carcinoma incidence; 45 cases of patients with gastric cancer in 21 patients (46.7%) SOCS-1 gene was methylated CpG island, adjacent tissues of 2 patients (11.1%), while 10 cases of normal gastric mucosa were not found SOCS-1 gene CpG island methylation, indicating that SOCS-1 gene methylation may be involved in the progression of gastric cancer. Studies have shown that methylation of some genes, such as, E-cadherin and p16INK4a, and age-related[4]. Our study found that gastric tissue from healthy young people are not found hypermethylated SOCS-1 gene. In our study, SOCS-1 methylation and reduction of SOCS-1 expression in gastric carcinoma and lymph node metastasis, and tumor staging, indicating that SOCS-1 may be involved in the progress of the missing lymph node metastasis and tumor. Recent studies have shown that, JAK / STAT signaling pathway with many tumor occurrence and development are closely related, JAK / STAT signaling pathway may be a variety of cytokines and growth factors activate, resulting in transcriptional activation of target genes[5-7]. The SOCS family of proteins that can be activated STAT proteins and down JAK / STAT signaling pathway. SOCS-1 gene silencing may lead to JAK / STAT signaling pathway activation and persistence cmyc, c-fos and other JAK / STAT signaling pathway downstream genes highly expressed, and thus make oncogenes or abnormal expression of growth-related genes, leading to tumorigenesis. SOCS-1 gene is located on human chromosome 16p13.3, cytokines such as IL-2, IL-4, IL-13, etc. can induce the expression of SOCS-1, which could hinder the activity of JAK, leading to JAK / STAT signaling pathway termination or attenuation thereby negatively regulate the expression of cytokines[8]. DNA methylation such inactivation SOCS-1 chain and the interference negative feedback such gastric cancer cells highly sensitive to cytokines and growth factors. IL-6 is a gastric cancer cell survival and growth factors necessary for cell and serum levels of IL-6 is closely related to gastric cancer disease state, and therefore, SOCS-1 deletion in gastric cancer cells may enhance IL-6 signal reactivity by this maintain the malignant proliferation of gastric cancer cells and expansive growth[9-11]. SOCS-1 gene was considered a candidate tumor suppressor gene, because the

expression of SOCS-1 translocation can inhibit liver cancer cell proliferation and anchorage-independent growth, while those originally hepatoma cells by DNA methylation make SOCS-1 expression in silence[12]. Studies have shown that JAK / STAT signaling pathway activation make the target gene c-fos expression, and metastatic tumors. Thus, DNA methylation causes inactivation of SOCS-1 that JAK / STAT signaling pathway activation, particularly IL-6 signal transduction pathway, and then make the c-fos activation, leading to the occurrence of gastric cancer development and metastasis. Thus, SOCS-1 gene methylation may be a good reflection of tumor progression and metastasis of molecular markers, and as an early diagnosis of gastric cancer. Due to loss of methylation-mediated living is a potentially reversible phenomenon, so through the expression of SOCS-1 in the treatment of drug-induced demethylation can control the onset and progression of gastric cancer. In conclusion, we found that DNA methylation through can cause apparent SOCS-1 gene silencing, and methylation levels in gastric cancer with a large proportion of hypermethylation leads to inactivation of SOCS-1 may be involved in gastric carcinogenesis, progression and transfer. More research is needed to confirm the SOCS-1 inactivation process involved in gastric tumor biological effects, our research shows that SOCS-1 methylation may be effective biomarkers discovery of a gastric cancer metastasis as well as the assessment of progress, SOCS-1 gene hypermethylation makes the JAK / STAT signaling pathway, inhibition of this pathway may be an important breakthrough in the treatment of gastric cancer.

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