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GASTRIC CANCER

Mechanism and pathobiologic implications of CHFR promoter methylation in gastric carcinoma

Yu-Jia Gao, Yan Xin, Jian-Jun Zhang, Jin Zhou

Yu-jia Gao, Yan Xin, The Fourth Laboratory of Cancer Institute & Department of Tumor Pathology of General Surgery Institute, The First Affiliated Hospital of China Medical University, Shengyang 110001, Liaoning Province, China
Jian-Jun Zhang, Jin Zhou, Department of Surgery and Internal Medicine, Liaoning Tumor Hospital, Shengyang 110042, Liaoning Province, China
Author contributions: Gao YJ, Xin Y contributed equally to this work; Gao YJ and Xin Y designed and performed the research; Gao YJ and Xin Y were responsible for data management and analysis; Zhang JJ and Zhou J assisted with the data collection and provided specimens; Gao YJ wrote the manuscript.
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Correspondence to: Yan Xin, The Fourth Laboratory of Cancer Institute & Department of Tumor Pathology of General Surgery Institute, The First Affiliated Hospital of China Medical University, Shengyang 110001, Liaoning Province, China. yxin@mail.cmu.edu.cn
Telephone: +86-24-83282351 Fax: +86-24-83282351
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Abstract

AIM: To investigate the aberrant methylation of CHFR promoter in human gastric cancer (GC) and its impact on the expression of *CHFR* mRNA and protein, as well as its correlation with clinical and histological features of human GC.

METHODS: Methylation-specific polymerase chain reaction (MSPCR) was used to detect the methylation status of CHFR promoter in 20 primary GC samples and paired normal gastric mucosa. The *CHFR* mRNA and protein expressions were investigated both by RT-PCR and by Western blotting. The CHFR protein expression in 69 GC samples was immunohistochemically examined.

RESULTS: The DNA methylation of the *CHFR* gene was found in 9 of the 20 GC samples (45%) and the down-regulation of *CHFR* mRNA and protein was significantly associated with the methylation status of the *CHFR* gene (P = 0.006). In 20 samples of corresponding non-neoplastic mucosa, no DNA methylation of the CHFR gene was detected. The *CHFR* gene methylation in poorly differentiated GC samples was significantly higher than that in well-differentiated GC samples (P = 0.014). Moreover, the negative CHFR protein expression rate in paraffin-embedded GC samples was 55.07% (38/69), the positive rate in poorly differentiated GC samples was 36.73% (18/49), which was significantly lower than 65.00% (13/20) in well-differentiated GC samples (χ^2 = 4.586, P = 0.032).

CONCLUSION: Aberrant methylation of the *CHFR* gene may be involved in the carcinogenesis and development of GC, and is the predominant cause of down-regulation or loss of *CHFR* mRNA or protein expression. As aberrant methylation of CHFR promoter is correlated with tumor differentiation, it may help to predict the prognosis of GC and *CHFR* may become a novel target of gene therapy for GC in the future.

Key words: *CHFR* gene; Gastric carcinoma; DNA methylation

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INTRODUCTION

Gastric cancer (GC) is one of the most common malignancies worldwide[1]. As other malignant tumors, gastric carcinogenesis is a pathological process involving multiple genes and steps. The relevant genes are mainly oncogenes, tumor suppressor genes, and DNA mismatch repair genes. Tumor suppressor genes may lose their functions by gene mutation, loss of heterozygosity and methylation of promoters. Methylation is an epigenetic modification whereby the gene activity is controlled by adding methyl groups (CH3) to specific cytosines of the DNA. This control mechanism is important during mammalian embryonic development, and has received increasing attention in

carcinogenesis research[2]. Aberrant DNA methylation can change the chromosomal structure and DNA stability, cause abnormalities of gene expression, and affect proliferation and differentiation of tumors[3]. Hypermethylation in the promoter region, as a key factor for carcinogenesis, causes silencing of suppressor genes[4,5]. *CHFR* (checkpoint with FHA and RING finger) is a mitotic checkpoint gene that is localized at chromosome 12q24.33. *CHFR* encodes a protein with FHA and RING finger domains that governs transition from prophase to metaphase in the mitotic checkpoint pathway. In cellular response to mitotic stress by microtubule inhibitors, *CHFR* activation delays chromosome condensation during prophase and increases the cells' ability to survive the stress[6]. *CHFR* prevents errors in chromosome segregation that can lead to neoplasia. Recently, some studies showed that *CHFR* is an important tumor suppressor gene and its encoding product is a ubiquity ligase of Plk1[7-10]. Plk1 regulates both Wee1 kinase and Cdc25C phosphatase, which in turn control the Cdc2 kinase activity at the G2 to M transition. The *CHFR* gene can ubiquitinate and degenerate the Plk1, which prevents cells from entering prophase and metaphase. *CHFR* is ubiquitously expressed in normal human tissues while loss of *CHFR* expression has been observed in human tumors, in which it fails to prevent proliferation of abnormal cells from G2 to M phase, and abnormal differentiation and proliferation of cells occurs[11]. Moreover, CHFR protein, comprised of fork head- associated FHA and RING-finger (RF) domain, is frequently down-regulated in human colon cancer and GC (up to 50%). Loss of *CHFR* mRNA expression is a consequence of promoter methylation, suggesting that it plays a tumor suppressor role in gastrointestinal carcinogenesis. The checkpoint function of the FHA domain of *CHFR* is a core component of anti-proliferating properties against gastrointestinal carcinogenesis[12]. GC is the second most common cause of cancer-related death in Asia. Although surgery is the standard treatment for this disease, early detection and treatment are the only way to reduce its mortality[13].

This study was performed to assess the methylation of CHFR promoter in Chinese GC tissue samples, its impact on gene expression, and its correlation with the clinical and pathobiological characteristics of GC.

MATERIALS AND METHODS

Tissue samples and DNA extraction

We studied GC samples and adjacent normal mucosa from 20 patients who underwent surgical resections at the Department of Surgery of Liaoning Tumor Hospital (Shengyang, China) from March to September 2007. None of these patients received chemotherapy or radiotherapy before surgery. Informed consent for use of the samples was obtained from each patient before surgery. All tissue samples were confirmed by histopathology. The samples were placed in liquid nitrogen immediately and then stored at -80℃ until analysis. For immunohistochemical analysis, we used archival formalin-fixed, paraffin-embedded tissues from 69 GC patients and paired normal gastric mucosa, which were obtained from the First Affiliated Hospital of China Medical University during December 2003 to May 2004. Age and sex of the patients, tumor size, differentiation degree, Borrman type, depth of tumor invasion and status of lymph node metastasis were obtained from the histopathological reports of these patients. We attributed highly or moderately differentiated adenocarcinoma to "well differentiated", and attributed adenocarcinoma, mucinous carcinoma and signet cell carcinoma to "poorly differentiated". High molecular weight genomic DNA was extracted using the TIANamp genomic blood/cell/tissue genomic DNA kit (TIANGEN Biotech, Beijing), according to the manufacturer's instructions.

Bisulfite modification and methylation-specific poly merase chain reaction (MSPCR)

Sodium bisulfite treatment of DNA converted all unmethylated cytosines to uracils, but the level of methylated cytosines was unaffected. Briefly, 2 μg aliquots of genomic DNA was denatured by adding 6 μL freshly prepared 3 mol/L NaOH and incubating the solution at 37℃ for 10 min. For complete denaturation, the samples were incubated at 95℃ for 1 min and subsequently cooled on ice. Bisulphate solution was prepared by dissolving 8.1 g sodium bisulphate in 16 mL H_2O, adding 30 μL 10 mmol/L hydrochinone solution and adjusting the pH to 5.0 with 520 μL 3 mol/L NaOH. Bisulphate solution (0.5 mL) was mixed with the denatured DNA, overlaid with mineral oil, and incubated at 50℃ for 17.5 h in a water bath in the dark. DNA was recovered using the Wizard DNA clean-up system (Promega, Madison, WI, USA) and eluted in 100 μL H_2O. Following this, 11 μL 3 mol/L NaOH was added and the sample was incubated for 15 min at 37℃. The solution was then neutralized by adding 110 μL 16 mol/L NH_4OAC (pH 7.0). DNA was ethanol-precipitated, washed with 70% ethanol, dried and resuspended in 50 μL distilled H_2O. Bisulphate-treated DNA, as a template for MSPCR, was used in each of the PCR assays. Amplification was carried out in a 25 μL reaction volume containing 2 μL 1 × PCR buffer, 2.0 mmol/L $MgCl_2$, 2.5 mmol/L dNTPs, 50 mg/mL DMSO, 10 μmol/L each primer, 50 ng DNA template, 2 U hot start Taq polymerase (Finzymes OY, Finland). After heating at 95℃ for 5 min, PCR was performed in a thermal cycler for 40 cycles of denaturation at 95℃ for 45 s, annealing at 51℃ (CHFR-UMS) or at 55℃ (CHFR-MS) for 45 s and extension at 72℃ for 45 s. Distilled water without DNA was used as a negative control. The PCR products were separated on 3.0% agarose gels. The following primer sets were used: CHFR M forward (5'-TTTT AATATAATATGGCGTCGATC-3'), a CHFR M reverse (5'-AACGACAACTAAAACGAAACCG-3') for methylated CHFR sequences, which could amplify a 141-base pair product. CHFR U forward (5'-GTTT

TAATATAATATGGTGTTGATT-3') and CHFR U reverse (5'-AAAACAACAACTAAAACAAA CCA-3') for unmethylated CHFR sequences, which could amplify a 144-base pair product as described previously[14].

Reverse transcription-PCR (RT-PCR)

Expression of the *CHFR* gene was analyzed by RT-PCR. Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. First-strand cDNA was generated using a first strand cDNA synthesis kit (Qiagen, German), then amplified by a primer set that is specific for the *CHFR* gene. The primer sequences are CHFR S (sense):5'-TAAAGGAAGTGGTCCCTCTGTG-3' and CHFR AS (anti-sense): 5'-GGTTTGGGCATTTCTA CGC-3', which resulted in a DNA product of 205 bp. The PCR amplification consisted of 1 cycle at 95℃ for 5 min, 35 cycles at 95℃ for 30 s, at 58℃ for 45 s, and at 72℃ for 1 min, and 1 cycle at 72℃ for 6 min. The expression of β-actin was used as a control to confirm the success of RT-PCR using the following primer pair: 5'-AGTTGCGTTACACCC TTTCTTG-3' (forward) and 5'-TCACCTTCACCGTTCCAGTTT-3' (reverse). The PCR products were separated by 2% agarose gel electrophoresis, stained with ethidium bromide, and visualized under UV light. Electrophoresis strips were analyzed by BANDSCAND 5.0 software and the optical density ratio of target mRNA to β-actin served as an index for statistical analysis. If the relative optical density value of *CHFR* mRNA expression was decreased more than 50% compared with paired normal gastric mucosa, it was defined as down-regulation of expression. No expression was regarded as loss of mRNA expression.

Western blotting

Tumor and control tissue samples were homogenized for extract preparations in an ice-cold mild lysis buffer containing 10 mL/L nonidet P-40, 0.15 mol/L NaCl, 0.01 mol/L sodium phosphate (pH 7.2), 2 mmol/L EDTA, 50mmol/L sodium fluoride, 0.2 mmol/L sodium vanadate, and 1 μg/mL aprotinin. The tissue homogenates were centrifuged at 20 000 r/min for 15 min and supernatants were collected. Protein density was determined by Coomassie brilliant blue, and then 12% SDS polyacrylamide gel electrophoresis was performed. Separated proteins were then transferred onto nitrocellulose membranes, which were blocked in 50 g/L nonfat milk in TBST (TBS buffer containing 1 g/L Tween 20) for 2 h at room temperature, incubated in primary antibodies specific for mouse CHFR (1:400 dilution) for 2 h, washed and probed with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. Immunoreactive bands were visualized by enhanced chemiluminescence (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The specific bands were quantified by BANDSCAN 5.0 software and the optical density ratio of target protein to β-tubulin served as an index for statistical analysis. If the relative optical density value of CHFR protein expression was decreased more than 50% compared with paired normal gastric mucosa, it was defined as down-regulation of expression. No expression was regarded as loss of protein expression.

Immunohistochemical staining

Tissue chips of GC, precancerous lesions and normal gastric mucosa were immunohistochemically stained by the Envision method. Immuno-bridge kits, mouse anti-human CHFR monoclonal antibody (diluted at 1:75) were bought from Abnova Company. All steps were accomplished in accordance with the instructions. PBS (0.01 mol/L, pH7.4) was used instead of specific antibodies for negative control. Immunohistochemical staining was graded as positive if the staining signals of CHFR protein were yellow brown granules and located in cytoplasm. For each sample, two representative high power fields were examined. The average positive rate was assessed by the percent of positive cells in the totally counted 100 cells from two representative high power fields. Positive cells ≤ 20% of the totally counted cells were defined as negative while positive cells > 20% of the totally counted cells as positive.

Statistical analysis

The data were processed by SPSS 13.0 statistical software. Quantitative data were expressed as mean ± SD. Data were analyzed by Fisher's exact test and chi square test, independent sample t-test and Spearman rank related test. $P < 0.05$ was considered statistically significant.

RESULTS

Aberrant methylation of CHFR gene in GC tissue samples

The representative results of MSPCR for the *CHFR* gene promoter in GC samples are shown in Figure 1 and Table 1. DNA methylation of the *CHFR* gene was detected in 9 (45%) of the 20 GC samples. By contrast, no methylation was detected in the corresponding normal gastric mucosa from these same patients. The difference in GC tissue and normal gastric mucosa samples was significant ($P < 0.001$). A significant difference was observed in the tumor samples ($P = 0.014$), indicating that poorly differentiated GC is more frequently methylated than well-differentiated GC. However, aberrant methylation of the *CHFR* gene in human GC was not significantly correlated with other clinicopathological factors such as gender, age, tumor size, Borrman type, depth of tumor invasion, and status of lymph node metastasis.

Aberrant expression of CHFR mRNA in GC tissue samples

As shown in Table 2, *CHFR* mRNA expression was down-regulated in GC tissue samples (0.2186 ± 0.2113) compared with normal gastric mucosa (0.7020 ± 0.2163) and the difference was significant ($t = 7.148$, $P < 0.0001$).

Table 1 Clinicopathological features of CHFR promoter methylation in GC

Variable	*n*	Methylated	Unmethylated	Methylated rate (%)	*P* value
Total	20	9	11		
Age (yr)					
≤ 50	6	3	3	50.00	1.000
> 50	14	6	8	42.86	
Gender					
Female	12	5	7	41.67	1.000
Male	8	4	4	50.00	
Gastric cancer					
Tumor size (cm)					
< 5.0	9	5	4	55.56	0.653
≥ 5.0	11	4	7	36.36	
Borrman type					
Ⅰ + Ⅱ	10	4	6	40.00	1.000
Ⅲ + Ⅳ	10	5	5	50.00	
Differentiation degree					
Well	6	0	6	0.00	0.014
Poorly	14	9	5	64.29	
Invasive depth					
Within Muscle layer	5	2	3	40.00	1.000
Penetratingmuscle layer	15	7	8	46.67	
Lymph node metastasis					
Positive	12	6	6	50.00	0.670
Negative	8	3	5	37.50	

Table 2 Clinicopathological features of *CHFR* mRNA and protein expression in GC

Variable	*n*	Relative expression density of *CHFR* mRNA	*t*	*P*	Relative expression density of CHFR protein	*t*	*P*
Age (yr)			1.189	0.176		0.965	0.347
≤ 50	6	0.1400 ± 0.1572			0.3300 ± 0.3809		
> 50	14	0.2800 ± 0.2187			0.2064 ± 0.1989		
Gender			0.608	0.551		0.648	0.525
Female	12	0.1942 ± 0.2179			0.2125 ± 0.2618		
Male	8	0.2537 ± 0.2094			0.2913 ± 0.2734		
Gastric cancer							
Tumor size (cm)			0.872	0.395		1.346	0.195
< 5.0	9	0.1722 ± 0.1780			0.3289 ± 0.3360		
≥ 5.0	11	0.2555 ± 0.2364			0.1736 ± 0.1678		
Borrman type			0.797	0.436		0.208	0.838
Ⅰ + Ⅱ	10	0.2560 ± 0.2312			0.2310 ± 0.2772		
Ⅲ + Ⅳ	10	0.1800 ± 0.1934			0.2560 ± 0.2602		
Differentiation degree			3.276	0.004		5.162	0.001
Well	6	0.4106 ± 0.1574			0.5447 ± 0.2573		
poorly	14	0.1364 ± 1.1772			0.1143 ± 0.1224		
Invasive depth			0.296	0.907		1.243	0.230
Within muscle layer	5	0.2480 ± 0.1620			0.3678 ± 0.3346		
Penetrating muscle layer	15	0.2147 ± 0.2320			0.2020 ± 0.2320		
Lymph node metastasis			1.892	0.075		1.318	0.204
Positive	12	0.1500 ± 0.1839			0.1817 ± 0.2353		
Negative	8	0.3200 ± 0.2190			0.3363 ± 0.2879		

Among them, *CHFR* mRNA expression in 14 poorly differentiated GC tissue samples (0.1364 ± 1.772) was significantly lower than that (0.4106 ± 0.1574) in 6 well-differentiated GC samples (t = 3.276, P = 0.004). The representative RT-PCR results for the *CHFR* mRNA expression in GC samples are shown in Figure 2.

CHFR protein expression levels in GC tissue samples

Western blotting analysis showed that the down-regulation and loss rate of the CHFR gene-coded protein was 70.00% (14/20) in GC tissue samples. The difference in relative optical density value between GC tissue and normal gastric mucosa samples was significant (0.2435 ± 0.2620 *vs* 0.5955 ± 0.2196, t = 4.605, P < 0.0001). The level of CHFR protein expression in poorly differentiated GC tissue samples was significantly lower than that in well-differentiated GC tissue samples (t = 5.162, P = 0.001). The CHFR protein expression level was correlated with the other clinicopathological features of GC tissue samples and paired normal gastric mucosa samples (Table 2). The representative Western blotting results for the CHFR protein expression in GC samples

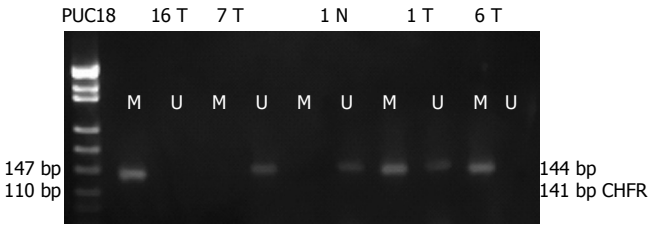

Figure 1 Representative results of MSPCR in human GC tissue samples. Lanes U and M: Products derived from unmethylated and methylated alleles, respectively. Methylation of the *CHFR* gene was detected in 16 and 6 poorly-differentiated adenocarcinoma tissue samples and 1 mucinous adenocarcinoma tissue sample, while unmethylation of the *CHFR* gene was detected in 7 well-differentiated adenocarcinoma tissue samples. PUC18: Marker; N: Normal gastric mucosa corresponding to tumors; T: Gastric cancer.

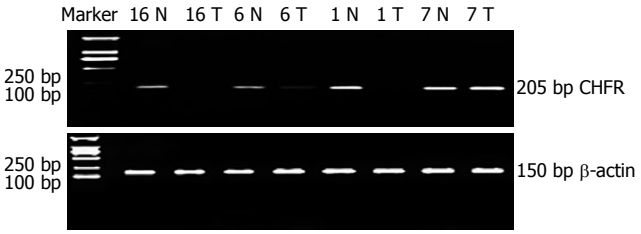

Figure 2 Representative results of RT-PCR in human GC tissue samples. β-actin was used as an internal control. *CHFR* mRNA expression level in 16 and 6 poorly differentiated adenocarcinoma tissue samples and 1 mucinous adenocarcinoma tissue sample was significantly lower than that in paired normal gastric mucosa samples from the same patients, while no difference was found in 7 well-differentiated adenocarcinoma tissue samples. Marker, D2000: Marker; N: Normal gastric mucosa corresponding to tumors; T: Gastric cancer.

are shown in Figure 3.

In addition, the CHFR protein expression in 69 GC tissue samples was analyzed by immunohistochemical staining of paraffin-embedded sections. Negative staining was observed in 55.07% of GC tissue samples (38/69). Positive staining was observed in 36.73% of poorly differentiated GC tissue samples (18/49), which was significantly lower than 65.00% (13/20) of well-differentiated GC tissue samples ($\chi^2 = 4.586$, $P = 0.032$). However, the CHFR protein expression in human GC tissue samples was not correlated to the other clinicopathological factors such as gender, age, tumor size, Borrman type, depth of tumor invasion and status of lymph node metastasis. The example of immunohistochemical staining is shown in Figure 4.

Correlation of aberrant CHFR methylation with mRNA and protein expression level

The level of *CHFR* mRNA and protein expression in 9 GC tissue samples with CHFR methylation was down-regulated or lost. The level of mRNA and protein expression was down-regulated only in 5 of the 11 GC tissue samples with an unmethylated *CHFR* gene. The *CHFR* mRNA and protein expression was inversely correlated with promoter methylation ($r = 0.592$, $P = 0.006$).

DISCUSSION

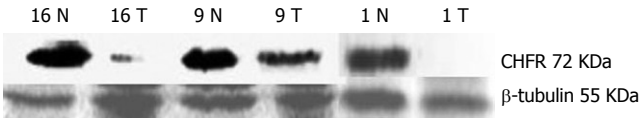

Figure 3 Representative results of Western blot in human GC tissue samples. β-tubulin was used as an internal control. CHFR protein expression level in GS tissue samples was significantly lower than that in paired normal gastric mucosa samples. Aberrant methylation of CHFR and down-regulation or loss of *CHFR* mRNA expression were detected in 16 poorly differentiated adenocarcinoma tissue samples and 1 mucinous adenocarcinoma tissue sample, while positive protein expression of CHFR was detected in 9 moderately-differentiated adenocarcinoma tissue samples without CHFR methylation. N: Normal gastric mucosa corresponding to tumors; T: Gastric cancer.

It is well known that both carcinogenesis and tumor progression evolve from genetic and epigenetic alterations of several genes[15]. Epigenetic alteration refers to the heritable phenotypic alteration in the absence of DNA sequence changes, and DNA methylation is one of the extensively studied epigenetic alterations[16]. In human beings and other mammals, CpG island methylation is an important physiological mechanism. The inactivated X-chromosome in female silenced alleles of imprinted genes or inserted viral genes and repeat elements are inactivated through promoter methylation[17]. Aberrant CpG methylation is common in cancer development and may play an important role in the carcinogenic process[18-25]. Methylation changes occurring in cancer include global hypomethylation in genomic DNA and gene-specific promoter hypermethylation. Whereas global hypomethylation increases mutation rates and chromosomal instability, promoter hypermethylation usually results in transcriptional gene inactivation. Thus, promoter hypermethylation, by silencing anti-cell-proliferation genes, anti-apoptosis genes, anti-angiogenesis genes, DNA repair genes, and anti-metastasis genes, plays an important role in carcinogenesis[26,27]. Recent evidence indicates that epigenetic changes might "addict" cancer cells to altered signal-transduction pathways during the early stages of tumor development. Dependence on these pathways for cell proliferation or survival allows them to acquire genetic mutations in the same pathways, providing the cells with selective advantages that promote tumor progression[28]. Processes that regulate gene transcription are directly under the influence of genome organization. DNA methylation of CpGs constitutes an epigenetic mark generally correlated with transcriptionally silent condensed chromatin. Replication of methylation patterns by DNA methyltransferases maintains genome stability through cell division. Periodic, strand-specific methylation and demethylation occur during transcriptional cycling of the *pS2/TFF1* gene promoter activated by estrogens. DNA methyltransferases exhibit dual actions during these cycles and are involved in CpG methylation and active demethylation of 5mCpGs through deamination. Inhibition of this process precludes demethylation of the *pS2* gene promoter and its subsequent transcriptional activation. Cyclical changes in the methylation status of

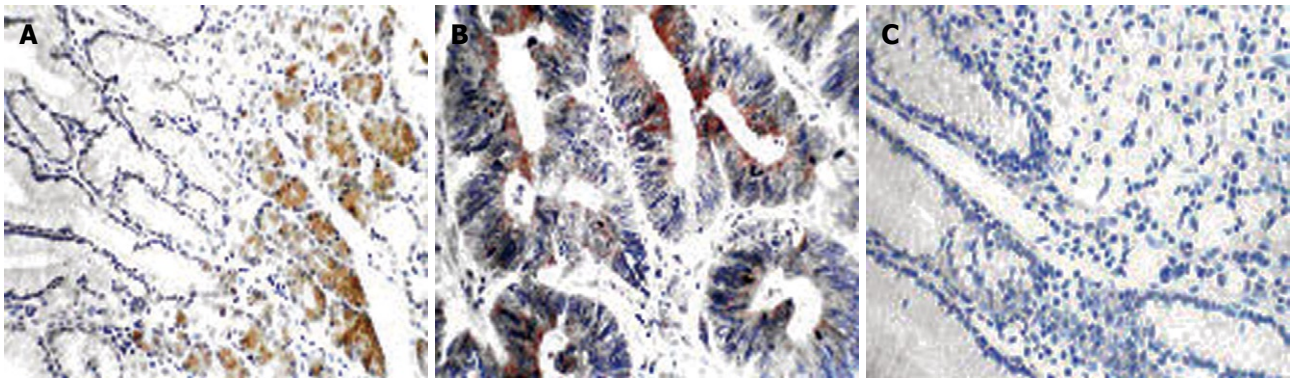

Figure 4 Immunohistochemical staining for CHFR protein expression in GC tissue samples and normal gastric mucosa samples. Positive expression of CHFR in normal gastric mucosa tissue samples (**A**), in well-differentiated adenocarcinoma tissue samples (**B**), and in signet-ring cell carcinoma tissue samples (**C**).

promoter CpGs may thus represent a critical event in transcriptional achievement[29].

However, aberrant methylation of CpGs is not a random event, but an event with gene-specific or tissue-specific differentiation. According to quantitative DNA methylation patterns at 4600 *Not* I sites and more than 150 differentially methylated regions in several C57BL/6J mouse tissue samples, comparative analysis between mice and human beings suggests that some, but not all, tissue-specific differentially methylated regions are conserved. A deeper understanding of cell-type-specific differences in DNA methylation might lead to a better illustration of the mechanisms behind tissue-specific differentiation in mammals[16].

As a tumor suppresser gene, aberrant methylation of CHFR promoter region associated with gene silencing has been reported in several primary tumors as followings[30-35].

The aberrant hypermethylation rate of CHFR was 12.3% (2/14) in cervical adenocarcinoma samples[30]. Thirty-six percent of patient samples showed a low or negative CHFR protein expression or staining. In addition, lack of CHFR detection was associated with increased tumor size and weakly correlated with estrogen receptor-negative tumors, suggesting that decreased CHFR expression results in the acquisition of many phenotypes associated with malignant progression, including accelerated growth rates, higher mitotic index, enhanced invasiveness, increased motility, greater aneuploidy, and amplified colony formation in soft agar, further supporting the role of *CHFR* as a tumor suppressor in breast cancer[31]. An aberrant methylation of the *CHFR* gene was detected in 25 out of 98 (26%) primary colorectal cancers and no methylation was detected in the corresponding normal tissue specimens[32]. In 46 patients with GC, 24 (52%) had aberrant CHFR methylation. By contrast, aberrant methylation was detected in only 2 samples (4%) of normal gastric mucosa and CHFR methylation status did not correlate with gender, sex, and clinicopathological features, such as tumor size, histological type, and stage. In cell lines, aberrant CHFR methylation correlated with the loss of mRNA expression, and treatment with the methyltransferase inhibitor 5-aza-dC induced re-expression of the gene, indicating that loss of CHFR expression due to aberrant methylation may be a cancer-specific event, which frequently occurs in primary GC[33]. CHFR staining was lost in 33% (57/174) of GC tissue samples, and there was a significant difference between staining in diffuse and intestinal histology. Loss of CHFR expression was found more commonly in the diffuse-type GC ($P = 0.001$), but no correlation was observed with age, location or tumor stage[34]. The aberrant methylation rate of CHFR was 41.1% (23/56) in GC samples. The mean age of patients with CHFR methylation was significantly higher than that of patients without CHFR methylation ($P = 0.040$). However, no significant correlation was observed with the other clinicopathologic factors[35].

In this study, the methylation rate of *CHFR* genes in GC tissue samples was significantly higher than that in paired normal gastric mucosa, suggesting that aberrant methylation of *CHFR* gene may be involved in carcinogenesis and development of GC. In addition, a significant difference was observed in GC ($P = 0.014$), indicating that poorly differentiated GC is more frequently methylated than well-differentiated GC and that aberrant methylation of the *CHFR* gene may participate in histological differentiation of GC and is associated with tumor malignant behavior. Its exact mechanism needs to be further studied. However, aberrant methylation of the *CHFR* gene in human GC was not significantly correlated with other clinicopathological factors such as gender, age, tumor size, Borrman type, depth of tumor invasion and status of lymph node metastasis. Moreover, the *CHFR* mRNA or protein expression level in 9 GC tissue samples with CHFR methylation was down-regulated or lost, while the level of the mRNA or protein expression was down-regulated only in 5 of 11 GC tissue samples with an unmethylated *CHFR* gene. These findings show that aberrant methylation of CHFR promoter in GC tissue samples is the main cause of down-regulation or loss of its mRNA or coded protein expression. On the other hand, CHFR staining was lost in 55.07% (38/69) of GC tissue samples, and there was a significant difference between staining in poorly differentiated and well-differentiated GC tissue samples. Because such a loss

was found more commonly in GC tissue samples, CHFR probably acts as a tumor suppressor in development of GC.

In conclusion, aberrant methylation of the *CHFR* gene is a frequent event in human GC and the principle mechanism underlying gene silencing, down-regulation or loss of CHFR. Since aberrant methylation of *CHFR* gene is closely correlated with tumor pathobiological behavior, the detection of the *CHFR* gene may be helpful in predicting the prognosis of GC and *CHFR* may become a novel target of gene therapy for GC in the future.

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COMMENTS

Background

CHFR is a novel tumor suppressor gene and its encoding product is a ubiquitous ligase of Plk1. In cellular response to mitotic stress induced by microtubule inhibitors, CHFR activation delays chromosome condensation during prophase and increases the cells' ability to survive the stress. CHFR prevents errors in chromosome segregation that can lead to neoplasia. CHFR is ubiquitously expressed in normal human tissues while loss of CHFR expression due to aberrant methylation has been observed in human tumors, suggesting that it may be involved in carcinogenesis and development of gastric cancer. To date, few studies have addressed whether aberrant methylation of CHFR promoter is a common event in gastric cancer (GC).

Research frontiers

The pathogenesis of GC is poorly understood. DNA methylation is an epigenetic modification. This control mechanism has received increasing attention in carcinogenesis research. As a tumor suppresser gene, aberrant methylation of CHFR promoter associated with gene silencing has been reported in several primary tumors including lung, esophageal, colorectal and hepatocellular cancers. In this study, we found that aberrant methylation of CHFR promoter played an important role in gastric carcinogenesis.

Innovations and breakthroughs

Our study showed that aberrant methylation of CHFR promoter was a frequent event in Chinese GC tissue samples and the principle mechanism underlying gene silencing, down-regulation or loss of CHFR. Aberrant methylation of CHFR promoter was closely correlated with tumor pathobiological behavior. Results of the present study may further our understanding of the molecular mechanism of DNA methylation, which is an epigenetic modification.

Applications

The data obtained from this study demonstrate that aberrant methylation of *CHFR* gene is a frequent event in human GC and the principle mechanism underlying gene silencing, down-regulation or loss of CHFR, suggesting that CHFR probably acts as a tumor suppressor in development of GC. Moreover, aberrant methylation of the *CHFR* gene was found to be closely correlated with tumor malignant behavior, indicating that detection of the *CHFR* gene may be helpful in predicting the prognosis of GC and CHFR may become a novel target of gene therapy for GC in the future.

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In this study, the authors showed that methylation of CHFR promoter was significantly increased in GC tissue samples and was higher in poorly differentiated GC tissue samples than in well-differentiated GC tissue samples. Protein expression was found to be inversely correlated with promoter methylation. These findings suggest that aberrant mathyltion of the *CHFR* gene may be involved in the development of gastric carcinoma. This paper is original and informative.

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