DNA Methylation of *CHFR* is not a Predictor of the Response to Docetaxel and Paclitaxel in Advanced and Recurrent Gastric Cancer

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Abstract. *Background: Cell cycle checkpoint dysfunction is often associated with sensitivity to chemotherapeutic agents. In this study, the question of whether DNA methylation of CHFR, a mitotic checkpoint gene, can predict the response of advanced and recurrent gastric cancers (GCs) to docetaxel or paclitaxel was examined. Materials and Methods: In a retrospective study, 41 patients with GC treated with paclitaxel alone (n=12) or a combination of docetaxel and S-1(tegafur, 5-chloro-2, 4-dihydroxypyridine, potassium oxonate) (n=29) were studied. The DNA methylation status of the CHFR gene was examined by combined bisulfite restriction analysis of DNAs from 41 GC tissues and the methylation status was compared to their sensitivity to chemotherapy. The levels of CHFR mRNA were measured by quantitative reverse transcription-PCR. Results: DNA methylation of CHFR was found in 15 (36.6%) out of the 41 GC samples and the levels of CHFR mRNA were associated with the methylation status of CHFR (p=0.034). In 41 samples of corresponding non-neoplastic mucosae, no DNA methylation of CHFR was detected. Among 12 patients treated with paclitaxel alone, only 1 (20.0%) of the 5 patients with CHFR methylation had a partial response (PR) to paclitaxel, whereas 3 (42.9%) of the 7 patients without CHFR methylation had a PR to paclitaxel (p=0.836). In 29 patients treated with a combination of S-1 and docetaxel, there was no clear association between the CHFR methylation status and response to chemotherapy (p=0.092). Conclusion: We conclude that the DNA methylation of CHFR alone cannot predict the response of advanced and recurrent GC to docetaxel or paclitaxel. Both paclitaxel and docetaxel may be effective for treatment of GC even if CHFR is expressed.*

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Gastric cancer (GC) is one of the most common cancers worldwide. Despite improvements in cancer diagnosis and therapy, many patients are still diagnosed in the late stages of the disease and recurrent disease is often found, even after curative surgery. Until recently, GC was considered a poorly chemo-responsive cancer; however, several clinical trials have shown that some chemotherapeutic agents are quite effective against GC.

Docetaxel and paclitaxel, both of which are microtubule inhibitors, are semi-synthetic toxoids with a broad spectrum of activity against transplanted tumors *in vivo*. S-1 is a novel oral fluorouracil antitumor drug that contains a combination of three pharmacological agents: tegafur, which is a 5-fluorouracil (5 FU) prodrug; 5-chloro-2, 4-dihydroxypyridine, which inhibits the activity of dihydropyrimidine dehydrogenase; and potassium oxonate, which reduces the gastrointestinal toxicity of 5 FU. Combination therapy with docetaxel and S-1 showed a high degree of efficacy in patients with advanced and/or recurrent GC (1); however, chemotherapy is often hindered by the development of drug resistance (2). Although global gene expression analyses, such as cDNA microarray analyses, have identified many candidate genes potentially involved in the drug-resistant phenotype (3, 4), there are no useful and reliable markers for the prediction of chemosensitivity to docetaxel or paclitaxel.

A variety of genetic and epigenetic alterations are associated with GC (5, 6). The hypermethylation of CpG islands is associated with the silencing of several genes and

has been proposed as a mechanism for the inactivation of tumor suppressor genes and tumor-related genes in GC (7-12). The identification of methylated genes may be useful in cancer diagnosis and therapeutics and may provide insights into carcinogenesis. Several lines of evidence suggest that the checkpoint protein with FHA and ring finger domains (*CHFR*) gene is an early mitotic checkpoint that delays transition to the metaphase in response to mitotic stress (13-15). Cell cycle checkpoint dysfunction is often associated with sensitivity to chemotherapeutic agents (16, 17). A previous study of GC cell lines indicated that the DNA methylation of *CHFR* may be a good molecular marker for prediction of the response to microtubule inhibitor-type drugs (18). GC cell lines with methylation of *CHFR* are sensitive to microtubule inhibitors including docetaxel. Although DNA methylation of *CHFR* has been reported in several cancers, including GC (19, 20), the clinical utility of the methylation status of CHFR for the prediction of chemosensitivity has not been investigated in cancers, including GC.

In the present retrospective study, the relationship between the DNA methylation of *CHFR* and the response of advanced and recurrent GCs to chemotherapy with paclitaxel or docetaxel was studied.

Materials and Methods

Patients and tumor specimens. The cohort comprised 41 patients with GC referred to the Department of Surgical Oncology, Hiroshima University Hospital (Hiroshima, Japan). Forty-one GC tissue samples and 41 corresponding non-neoplastic mucosae samples from the 41 patients were analyzed for the DNA methylation of *CHFR*. Total RNA was available for 12 pairs of GC samples and the corresponding non-neoplastic mucosae samples to study the expression of *CHFR*. GC and corresponding non-neoplastic mucosae samples were obtained by resection or biopsy before the initiation of chemotherapy. The specimens from 12 of the 41 cases were frozen immediately and stored at –80˚C. The remaining 29 specimens were archival, formalin-fixed, paraffin-embedded tissues. It was confirmed microscopically that the tumor tissue specimens consisted mainly (>80%) of cancer tissue and that the non-neoplastic mucosae did not show any evidence of tumor cell invasion or significant inflammatory involvement. All the patients had pathologically determined inoperable or recurrent GC. Of the 41 patients, 12 were treated with paclitaxel alone, while the remaining 29 were treated with a combination of docetaxel and S-1.

Paclitaxel at a dose of 80 mg/m^2 was infused over 1 hour on days 1, 8 and 15, followed by a 1-week interval. For the combination therapy with S-1 and docetaxel, S-1 was administered orally at a dose of 80 mg/m^2 within 30 minutes of the morning and evening meals for 2 weeks, followed by a 1-week interval (1 cycle). Docetaxel (40 mg/m^2) in 100 ml of 0.9% saline was infused over 1 hour on the morning of day 1. This cycle of administration was repeated every 3 weeks, and the infusion was started at the same time as the S-1.

All 41 patients provided a medical history and had a physical examination including evaluation of their performance status, complete blood cell count (CBC), serum chemistry profile, creatinine clearance, urinalysis, electrocardiography, chest X-ray and computed tomography (CT) and/or magnetic resonance imaging at the time of enrollment. An upper GI series, gastrointestinal fiberscopy (GIF) and barium enema were performed if necessary. Baseline biological analysis (CBC, serum chemistry profile and urinalysis) and physical examination, including determination of weight and performance status, were performed at least once a week while the patients were undergoing treatment. Tumor markers, including CEA and CA19-9, were checked once each month.

The responses of the primary and metastatic lesions to treatment were assessed according to the World Health Organization criteria. The primary and metastatic lesions were evaluated by GIF, CT, ultrasonography and other radiographic examinations. Complete response (CR) was defined as the absence of all evidence of cancer for more than 4 weeks. Partial response (PR) was defined as at least a 50% reduction in the sum of the products of the perpendicular diameters of all the lesions for more than 4 weeks, without any evidence of new lesions or progression of existing lesions. No change (NC) was defined as less than a 50% reduction or less than a 25% increase in the sum of the products of the perpendicular diameters of all lesions without any evidence of new lesions. Progressive disease (PD) was defined as more than a 25% increase in more than 1 lesion or the appearance of new lesions. Of the 12 patients treated with paclitaxel alone, none showed CR, 4 showed PR, 5 showed NC and 3 showed PD. Of the 29 patients treated with the combination of docetaxel and S-1, none showed CR, 19 showed PR, 4 showed NC and 6 showed PD.

Because written informed consent had not been obtained for the use of some samples, all identifying information was removed from all the samples before analysis to protect patient privacy. This procedure is in accordance with the Ethical Guidelines for Human Genome/Gene Research enacted by the Japanese Government.

Genomic DNA extraction and combined bisulfite restriction analysis (COBRA). Genomic DNAs from frozen tissue samples were extracted with a Genomic DNA Purification Kit (Promega, Madison, WI, USA). For DNA extraction from the archival, formalin-fixed, paraffin-embedded tissue samples, GC samples and corresponding non-neoplastic samples were manually dissected from different sets of 10 serial, 10-μm-thick, formalin-fixed, paraffin-embedded tissue sections with a fine needle. The dissected samples were lysed by incubation in 200 mg/ml proteinase K at 55˚C for 3 days. Genomic DNA was purified by 3 rounds of phenol/chloroform extraction followed by ethanol precipitation. To examine the DNA methylation patterns, genomic DNA was treated with 3 M sodium bisulfite, as described previously (21). For analysis of the DNA methylation of the *CHFR* gene, COBRA (22) was performed with primers and a restriction enzyme, as described previously (18). The PCR products (15 μl) were loaded onto 8% non-denaturing polyacrylamide gels, stained with ethidium bromide and visualized under ultraviolet light.

RNA extraction and quantitative reverse transcription (RT)-PCR analysis. The total RNA was extracted with an RNeasy Mini Kit (Qiagen, Valencia, CA, USA) and 1 μg of total RNA was converted to cDNA with a First Strand cDNA Synthesis Kit (Amersham Biosciences, Piscataway, NJ, USA). PCR was performed with a SYBR Green PCR Core Reagents Kit (Applied Biosystems, Foster City, CA, USA). Real-time detection of the emission intensity of SYBR Green bound to double-stranded DNA was performed with an ABI PRISM 7700 Sequence Detection

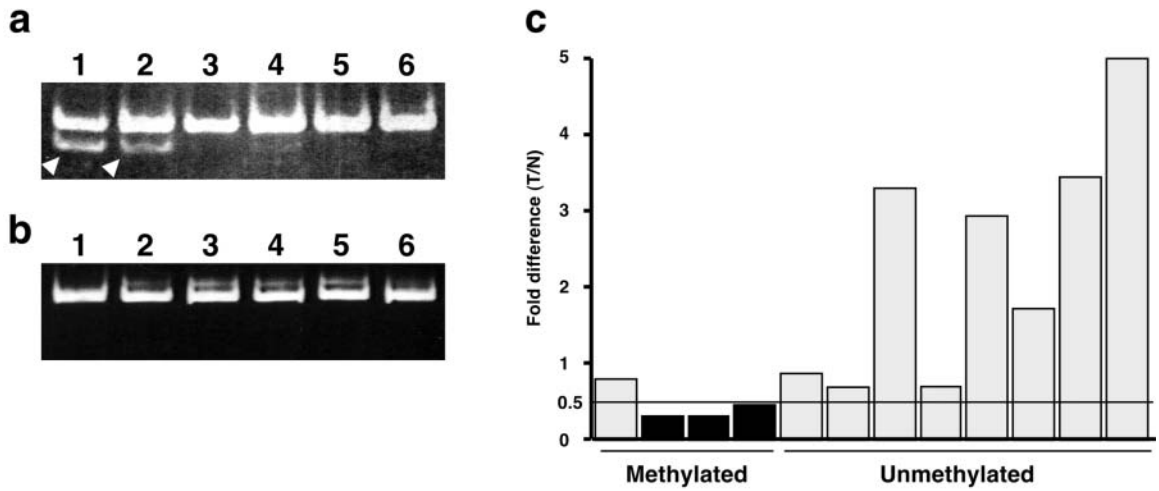

Figure 1. *COBRA and quantitative RT-PCR of CHFR in GCs and corresponding non-neoplastic gastric mucosae. (a) COBRA for CHFR in GCs. A methylated allele of CHFR was detected in samples 1T and 2T. (b) COBRA for CHFR in corresponding non-neoplastic gastric mucosae. Methylated alleles of CHFR were not detected (U=unmethylated PCR products, M=methylated PCR products, T=GC, N=corresponding non-neoplastic mucosae). (c) Quantitative RT-PCR analysis of CHFR. Fold-change indicates the ratio of CHFR mRNA level in GC (T) to that in corresponding non-neoplastic mucosae (N). Down-regulation of CHFR (T/N<0.5) was detected in 3 (75.0%) out of 4 GCs with methylation of CHFR, whereas down-regulation of CHFR was not detected in 8 GCs without CHFR methylation.*

System (Applied Biosystems), as described previously (23). The *CHFR* primer sequences were 5'- CCT CAA CAA CCT CGT GGA AGC ATA C -3' and 5'- TCC TGG CAT CCA TAC TTT GCA CAT C -3'. *ACTB*-specific PCR products served as internal controls. The ratio of the *CHFR* mRNA levels between GC tissue (T) and corresponding non-neoplastic mucosae (N) was calculated. T/N ratios of less than 0.5 were considered to indicate a down-regulation of expression.

Statistical methods. The differences were statistically analyzed by the Chi-square test. *P* values of less than 0.05 were considered statistically significant.

Results

Relationship between DNA methylation of CHFR and response of GC to paclitaxel and the combination of docetaxel and S-1. To investigate the 5' CpG island methylation status of the *CHFR* gene in GC tissues, COBRA of 41 GC tissues was performed. Representative results are shown in Figure 1a. The overall results are shown in Table I. In total, DNA methylation of the *CHFR* gene was detected in 15 (36.6%) of the 41 GC samples. Methylation of *CHFR* was not associated with the patient's age or sex (data not shown). In the 12 patients treated with paclitaxel alone, the *CHFR* methylation status was not associated with the response to paclitaxel. Only 1 (20.0%) of the 5 patients with *CHFR* methylation showed a PR to paclitaxel, whereas 3 (42.9%) of the 7 patients without *CHFR* methylation showed a PR ($p=0.836$, Chi-square test) (Table II). In the 29 patients treated with a combination of docetaxel and S-1, there was no clear association between the *CHFR* methylation status and response to combination therapy ($p=0.092$, Chi-square test) (Table III). In the 41 samples of corresponding non-neoplastic mucosae, no DNA methylation of *CHFR* was observed (Figure 1b).

CHFR methylation status and mRNA expression levels in GC tissues. The question of whether the DNA methylation of *CHFR* is involved in the down-regulation of the *CHFR* gene was addressed next. Of the 41 GC cases, total RNA was available for 12 pairs of GC and corresponding non-neoplastic mucosae to study the expression of *CHFR*. The overall results of the quantitative RT-PCR analysis are shown in Figure 1c. A reduced expression of *CHFR* was found in 3 (25.0%) of the 12 GC cases. As shown in Table IV, down-regulation of *CHFR* mRNA was significantly associated with DNA methylation of the *CHFR* gene ($p=0.034$, Chi-square test).

Discussion

It has been suggested that inactivation of *CHFR* by DNA methylation is a predictor of sensitivity to microtubule inhibitors such as docetaxel and paclitaxel. GC cell lines in which *CHFR* is inactivated by DNA methylation show high sensitivity to microtubule inhibitors (18). In the present study, 12 patients with GC were treated with paclitaxel alone; however, there was no association between the DNA

Table I. *Summary of DNA methylation of CHFR and clinical characteristics.*

No.	Age	Sex	Therapy	Response	Response evaluation	*CHFR* methylation	*CHFR* expression
1	61	Male	Paclitaxel	PR[a]	Peritoneal dissemination	Md	N.E.[f]
9	73	Male	Paclitaxel	PR	Peritoneal dissemination	Ue	N.E.
8	76	Male	Paclitaxel	PR	Peritoneal dissemination	U	N.E.
10	69	Male	Paclitaxel	PR	Peritoneal dissemination, lymph node metastasis	U	N.E.
12	88	Male	Paclitaxel	NC[b]	Lymph node metastasis, primary GC	M	N.E.
2	77	Female	Paclitaxel	NC	Lymph node metastasis, primary GC	M	N.E.
15	34	Female	Paclitaxel	NC	Lymph node metastasis, primary GC	M	N.E.
5	61	Female	Paclitaxel	NC	Peritoneal dissemination	U	Unchanged
6	69	Female	Paclitaxel	NC	Lymph node metastasis	U	N.E.
16	74	Female	Paclitaxel	PD[c]	Peritoneal dissemination	M	Down
4	81	Male	Paclitaxel	PD	Liver metastasis	U	Unchanged
3	69	Male	Paclitaxel	PD	Peritoneal dissemination, lymph node metastasis	U	Unchanged
32	85	Male	Docetaxel + S-1	PR	Liver metastasis, primary GC	M	Down
17	54	Male	Docetaxel + S-1	PR	Peritoneal dissemination	M	Down
41	72	Male	Docetaxel + S-1	PR	Peritoneal dissemination	M	N.E.
38	67	Female	Docetaxel + S-1	PR	Lymph node metastasis	M	N.E.
26	75	Male	Docetaxel + S-1	PR	Lymph node metastasis	U	Unchanged
28	73	Male	Docetaxel + S-1	PR	Lymph node metastasis, peritoneal dissemination	U	Unchanged
19	63	Male	Docetaxel + S-1	PR	Liver metastasis	U	N.E.
22	53	Male	Docetaxel + S-1	PR	Liver metastasis	U	N.E.
30	79	Male	Docetaxel + S-1	PR	Liver metastasis	U	N.E.
39	72	Male	Docetaxel + S-1	PR	Liver metastasis	U	N.E.
33	78	Female	Docetaxel + S-1	PR	Liver metastasis, primary GC	U	N.E.
11	65	Male	Docetaxel + S-1	PR	Lymph node metastasis, primary GC	U	N.E.
31	75	Male	Docetaxel + S-1	PR	Lymph node metastasis, primary GC	U	N.E.
20	77	Female	Docetaxel + S-1	PR	Peritoneal dissemination	U	N.E.
25	71	Female	Docetaxel + S-1	PR	Peritoneal dissemination	U	N.E.
34	87	Male	Docetaxel + S-1	PR	Peritoneal dissemination, primary GC	U	N.E.
35	78	Male	Docetaxel + S-1	PR	Peritoneal dissemination, primary GC	U	N.E.
40	71	Female	Docetaxel + S-1	PR	Peritoneal dissemination, primary GC	U	N.E.
21	74	Male	Docetaxel + S-1	PR	Lymph node metastasis	U	N.E.
37	54	Male	Docetaxel + S-1	NC	Liver metastasis, primary GC	M	N.E.
18	76	Female	Docetaxel + S-1	NC	Lymph node metastasis	M	N.E.
23	40	Male	Docetaxel + S-1	NC	Lymph node metastasis	M	N.E.
29	69	Female	Docetaxel + S-1	NC	Bone metastasis	U	N.E.
7	62	Male	Docetaxel + S-1	PD	Liver metastasis	M	Unchanged
13	70	Male	Docetaxel + S-1	PD	Peritoneal dissemination	M	N.E.
14	74	Male	Docetaxel + S-1	PD	Peritoneal dissemination	M	N.E.
27	72	Male	Docetaxel + S-1	PD	Liver metastasis	U	Unchanged
24	66	Male	Docetaxel + S-1	PD	Liver metastasis	U	Unchanged
36	57	Male	Docetaxel + S-1	PD	Peritoneal dissemination, primary GC	U	Unchanged

[a]PR=partial response, [b]NC=no change, [c]PD=progressive disease, [d]M=methylated, [e]U=unmethylated, [f]N. E.=not examined.

methylation of *CHFR* and the response to paclitaxel. We confirmed that the DNA methylation of *CHFR* is associated with the down-regulation of *CHFR* expression. We also examined 29 GCs treated with a combination of docetaxel and S-1. Of these GCs, 19 showed a PR; however, it is unclear which antitumor drug was effective. However, S-1 and docetaxel were not effective in the remaining 10 GCs. *CHFR* methylation, which is thought to be associated with high sensitivity to docetaxel, was detected in 6 (60%) of these 10 GCs. Taken together, these findings indicate that the DNA methylation status of *CHFR* is not a good marker for the prediction of response to docetaxel or paclitaxel in GC.

There are several possible explanations for the inconsistencies between our current data and those of previous studies. First, we examined *CHFR* in tissue samples, whereas Satoh *et al.* examined *CHFR* in GC cell lines (18).

Table II. *Relationship between DNA methylation of CHFR and response to paclitaxel alone.*

	CHFR methylation status		
Response	Methylated	Unmethylated	*P* value[a]
Complete response	0 (0.0%)	0	0.836
Partial response	1 (25.0%)	3	
No change	3 (60.0%)	2	
Progressive disease	1 (33.3%)	2	

[a]Chi-square test (complete response plus partial response *vs.* no change plus progressive disease).

Table III. *Relationship between DNA methylation of CHFR and response to combination chemotherapy of docetaxel and S-1.*

	CHFR methylation status		
Response	Methylated	Unmethylated	*P* value[a]
Complete response	0 (0.0%)	0	0.092
Partial response	4 (21.1%)	15	
No change	3 (75.0%)	1	
Progressive disease	3 (50.0%)	3	

[a]Chi-square test (complete response plus partial response *vs.* no change plus progressive disease).

Table IV. *Relationship between DNA methylation of CHFR and expression of CHFR mRNA.*

	CHFR methylation status		
CHFR expression	Methylated	Unmethylated	*P* value[a]
Down-regulated	3 (100.0%)	0	0.034
Not down-regulated	1 (11.1%)	8	

[a]Chi-square test.

Second, we investigated the methylation of *CHFR* in primary GCs, but the response to chemotherapy was evaluated in metastatic lesions in many cases. Previous studies in a mouse model revealed that genomic stability is important for tumor progression (24); because *CHFR* encodes an early mitotic checkpoint protein, the inactivation of *CHFR* can cause genomic instability. Therefore, in metastatic GC lesions, *CHFR* may be reactivated, even though it is inactivated by DNA methylation in the primary lesion. Analyses of the DNA methylation of *CHFR* in metastatic lesions are needed to clarify this issue. Of the 12 cases treated with paclitaxel alone, 4 showed PR, while in 3 (75%) of these 4 cases, DNA methylation of *CHFR* was not detected. This suggests that paclitaxel is effective even if *CHFR* is expressed. Although paclitaxel and docetaxel show high degrees of efficacy in patients with advanced and/or recurrent GC (1), no patient had a CR in the present study. Taken together, these findings suggest that other molecules may be associated with the resistance to paclitaxel and docetaxel chemotherapy. G2/M checkpoint genes are thought to be associated with resistance to chemotherapy with microtubule inhibitors. We previously reported that Chk1 and Chk2, which are G2/M checkpoint proteins, are overexpressed in GC (25). The overexpression of Chk1 and Chk2 may contribute to the resistance to microtubule inhibitors. BUB1, which is also a G2/M checkpoint protein, is reported to be overexpressed in GC (26). Overexpression of these G2/M checkpoint proteins may underlie the resistance of GC to microtubule inhibitors.

In the present study, DNA methylation of *CHFR* was detected in 15 (36.6%) out of 41 GC samples, though it was not found in the corresponding non-neoplastic mucosae. In addition, DNA methylation was associated with down-regulation of *CHFR* expression in GC tissues, suggesting that DNA methylation of *CHFR* plays a major role in the transcriptional silencing of *CHFR* in GC. Inactivation of *CHFR* by DNA methylation may lead to chromosomal instability during GC.

In summary, we conclude that the DNA methylation status of *CHFR* alone cannot predict the response of advanced and/or recurrent GC to docetaxel or paclitaxel. However, both paclitaxel and docetaxel are effective for the treatment of GC even if *CHFR* is expressed. Our ongoing phase II study of combination therapy with docetaxel and S-1 for GC may clarify the effectiveness of microtubule inhibitors against GC.

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References

1 Yoshida K, Hirabayashi N, Takiyama W, Ninomiya M, Takakura N, Sakamoto J, Nishiyama M and Toge T: Phase I study of combination therapy with S-1 and docetaxel (TXT) for advanced or recurrent gastric cancer. Anticancer Res *24*: 1843-1851, 2004.

2 Longley DB and Johnston PG: Molecular mechanisms of drug resistance. J Pathol *205*: 275-292, 2005.
3 Lamendola DE, Duan Z, Yusuf RZ and Seiden MV: Molecular description of evolving paclitaxel resistance in the SKOV-3 human ovarian carcinoma cell line. Cancer Res *63*: 2200-2205, 2003.
4 Kikuchi T, Daigo Y, Katagiri T, Tsunoda T, Okada K, Kakiuchi S, Zembutsu H, Furukawa Y, Kawamura M, Kobayashi K, Imai K and Nakamura Y: Expression profiles of non-small cell lung cancers on cDNA microarrays: identification of genes for prediction of lymph-node metastasis and sensitivity to anti-cancer drugs. Oncogene *22*: 2192-2205, 2003.
5 Yasui W, Oue N, Ono S, Mitani Y, Ito R and Nakayama H: Histone acetylation and gastrointestinal carcinogenesis. Ann N Y Acad Sci *983*: 220-231, 2003.
6 Oue N, Hamai Y, Mitani Y, Matsumura S, Oshimo Y, Aung PP, Kuraoka K, Nakayama H and Yasui W: Gene expression profile of gastric carcinoma: identification of genes and tags potentially involved in invasion metastasis and carcinogenesis by serial analysis of gene expression. Cancer Res *64*: 2397-2405, 2004.
7 Jones PA and Baylin SB: The fundamental role of epigenetic events in cancer. Nat Rev Genet *3*: 415-428, 2002.
8 Ushijima T and Okochi-Takada E: Aberrant methylations in cancer cells: where do they come from? Cancer Sci *96*: 206-211, 2005.
9 Toyota M, Ahuja N, Suzuki H, Itoh F, Ohe-Toyota M, Imai K, Baylin SB and Issa JP: Aberrant methylation in gastric cancer associated with the CpG island methylator phenotype. Cancer Res *59*: 5438-5442, 1999.
10 Kaneda A, Kaminishi M, Yanagihara K, Sugimura T and Ushijima T: Identification of silencing of nine genes in human gastric cancers. Cancer Res *62*: 6645-6650, 2002.
11 Oue N, Motoshita J, Yokozaki H, Hayashi K, Tahara E, Taniyama K, Matsusaki K and Yasui W: Distinct promoter hypermethylation of p16INK4a CDH1 and RAR-beta in intestinal diffuse-adherent and diffuse-scattered type gastric carcinomas. J Pathol *198*: 55-59, 2002.
12 Rashid A and Issa JP: CpG island methylation in gastroenterologic neoplasia: a maturing field. Gastroenterology *127*: 1578-1588, 2004.
13 Scolnick DM and Halazonetis TD: Chfr defines a mitotic stress checkpoint that delays entry into metaphase. Nature *406*: 430-435, 2000.
14 Matsusaka T and Pines J: Chfr acts with the p38 stress kinases to block entry to mitosis in mammalian cells. J Cell Biol *166*: 507-516, 2004.
15 Yu X, Minter-Dykhouse K, Malureanu L, Zhao WM, Zhang D, Merkle CJ, Ward IM, Saya H, Fang G, van Deursen J and Chen J: Chfr is required for tumor suppression and Aurora A regulation. Nat Genet *37*: 401-406, 2005.
16 Bunz F, Hwang PM, Torrance C, Waldman T, Zhang Y, Dillehay L, Williams J, Lengauer C, Kinzler KW and Vogelstein B: Disruption of p53 in human cancer cells alters the responses to therapeutic agents. J Clin Invest *104*: 263-269, 1999.
17 Suzuki H, Itoh F, Toyota M, Kikuchi T, Kakiuchi H and Imai K: Inactivation of the 14-3-3 sigma gene is associated with 5' CpG island hypermethylation in human cancers. Cancer Res *60*: 4353-4357, 2000.
18 Satoh A, Toyota M, Itoh F, Sasaki Y, Suzuki H, Ogi K, Kikuchi T, Mita H, Yamashita T, Kojima T, Kusano M, Fujita M, Hosokawa M, Endo T, Tokino T and Imai K: Epigenetic inactivation of CHFR and sensitivity to microtubule inhibitors in gastric cancer. Cancer Res *63*: 8606-8613, 2003.
19 Mizuno K, Osada H, Konishi H, Tatematsu Y, Yatabe Y, Mitsudomi T, Fujii Y and Takahashi T: Aberrant hypermethylation of the CHFR prophase checkpoint gene in human lung cancers. Oncogene *21*: 2328-2333, 2002.
20 Honda T, Tamura G, Waki T, Kawata S, Nishizuka S and Motoyama T: Promoter hypermethylation of the Chfr gene in neoplastic and non-neoplastic gastric epithelia. Br J Cancer *90*: 2013-2016, 2004.
21 Herman JG, Graff JR, Myohanen S, Nelkin BD and Baylin SB: Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. Proc Natl Acad Sci USA *93*: 9821-9826, 1996.
22 Xiong Z and Laird PW: COBRA: a sensitive and quantitative DNA methylation assay. Nucleic Acids Res *25*: 2532-2534, 1997.
23 Kondo T, Oue N, Yoshida K, Mitani Y, Naka K, Nakayama H and Yasui W: Expression of POT1 is associated with tumor stage and telomere length in gastric carcinoma. Cancer Res *64*: 523-529, 2004.
24 Rudolph KL, Millard M, Bosenberg MW and DePinho RA: Telomere dysfunction and evolution of intestinal carcinoma in mice and humans. Nat Genet *28*: 155-159, 2001.
25 Shigeishi H, Yokozaki H, Oue N, Kuniyasu H, Kondo T, Ishikawa T and Yasui W: Increased expression of CHK2 in human gastric carcinomas harboring p53 mutations. Int J Cancer *99*: 58-62, 2002.
26 Shigeishi H, Oue N, Kuniyasu H, Wakikawa A, Yokozaki H, Ishikawa T and Yasui W: Expression of Bub1 gene correlates with tumor proliferating activity in human gastric carcinomas. Pathobiology *69*: 24-29, 2001.

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