DNA methyltransferase3a expression is an independent poor prognostic indicator in gastric cancer

Xue-Yuan Cao, Hong-Xi Ma, Yan-Hong Shang, Mei-Shan Jin, Fei Kong, Zhi-Fang Jia, Dong-Hui Cao, Yin-Ping Wang, Jian Suo, Jing Jiang

AIM: To explore the alteration of DNA methyltransferase expression in gastric cancer and to assess its prognostic value.

METHODS: From April 2000 to December 2010, 227 men and 73 women with gastric cancer were enrolled in the study. The expression of DNA methyltransferases (DNMTs), including DNMT1, DNMT3a and DNMT3b, in the 300 cases of gastric carcinoma, of which 85 had paired adjacent normal gastric mucus samples, was evaluated by immunohistochemistry using a tissue microarray. Serum anti-Helicobacter pylori (H. pylori) IgG was detected by enzyme-linked immunosorbent assay (ELISA). The relationships between the above results and the clinicopathological characteristics were analyzed. Their prognostic value was evaluated using the Cox proportional hazards model.

RESULTS: In gastric cancer, expression of DNMTs was mainly seen in the nucleus. Weak staining was also observed in the cytoplasm. Expression of DNMT1, DNMT3a and DNMT3b in gastric cancer was significantly higher compared to that in the paired control samples (60.0% vs 37.6%, 61.2% vs 4.7%, and 94.1% vs 71.8%, P<0.01). The overall survival rate was significantly higher in the DNMT3a negative group than in the DNMT3a positive group in gastric cancer patients (Log-rank test, P=0.032). No significant correlation was observed between DNMT1 and DNMT3 expression and the overall survival time (Log-rank test, P=0.289, P=0.347). Multivariate regression analysis indicated that DNMT3a expression (P=0.025) and TNM stage (P<0.001), but not DNMT1 (P=0.54) or DNMT3b (P=0.62), were independent prognostic factors in gastric cancer. H. pylori infection did not induce protein expression of DNMTs.

CONCLUSION: The results suggest that expression of DNMT3a is an independent poor prognostic indicator in gastric cancer. DNMT3a might play an important role in gastric carcinogenesis.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: DNA methyltransferase; Prognosis; Gastric cancer; Expression; Helicobacter pylori

Core tip: Up to now, few studies investigated the expression of DNA methyltransferases (DNMTs) and the
relationship between their expression and histopathology in gastric cancer. In this article, DNMT1, DNMT3a and DNMT3b expression has been investigated in 307 gastric cancer patients. The results suggest that expression of DNMT3a is an independent poor prognostic indicator in gastric cancer. DNA methylation plays essential roles in the development of gastric cancer.

Cao XY, MaHX, ShangYH, JinMS, KongF, JiaZF, CaoDH, WangYP, SuojJ, JiangJ. DNA methyltransferase3a expression is an independent poor prognostic indicator in gastric cancer. WorldJGastroenterol2014;20(25):8201-8208.Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i25/8201.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i25.8201

INTRODUCTION

Gastric cancer is one of the most common malignancies in Asian countries, and remains the second leading cause of cancer-related death worldwide. Over 70% of new cases and deaths occur in developing countries[3,4]. DNA methylation plays an essential role in normal development and maintenance of tissue-specific gene expression patterns. In human cancer cells, increased CpG island methylation, which mediates tumor suppressor gene silencing, and genomic DNA hypomethylation can lead to genomic instability. Gastric cancer progression involves genes and numerous steps, such as the over-expression of oncogenes and inactivation of tumor suppressor genes[5,6]. In gastric cancer, DNA methylation change is a key contributor to human oncogenesis. Aberrant DNA methylation in the promoter regions of genes, which leads to inactivation of tumor suppressor and other cancer-related genes in cancer cells, is the most well-defined epigenetic hallmark in gastric cancer[5,6]. Several studies have indicated that DNA methylation occurs in the cancerous and para-cancerous areas.

The methylation process is catalyzed by DNA methyltransferases (DNMTs), including DNMT1, DNMT3a, and DNMT3b. With the assistance of DNMTs, methyl groups are transferred to the C5 position of the cytosine and guanine dinucleotides by S-adenosylmethionine[7]. The genetic expression of these regions is inhibited by methylation. DNMTs catalyze the transfer of methyl groups to cytosine and also participate in or maintain methylation. Several studies suggest that DNMT genes are over-expressed in human cancer and during cellular transformation[6,8]. Recently, Kim et al[9] reported that DNMT3A mutations are an independent adverse prognostic factor in younger AML patients with normal cytogenetics. Up to now, few studies have investigated the expression of DNMTs and the relationship between their expression and histopathology in gastric cancer. Ding et al[10] and Yang et al[11] reported the clinical significance of the expression of DNMT proteins. Our previous studies have shown that SNPs of DNMT influenced Helicobacter pylori (H. pylori) infection and gastric atrophy in humans[14]. However, it is still lacking the clinical evidence about the association between expression of DNMT proteins and prognosis of gastric cancer.

It was considered that change of expression of DNMTs accompanies genome-wide hypomethylation and oncogenic hypomethylation or genetic hypermethylation, may leading to tumor suppression. Therefore, the purpose of this study was to evaluate the expression of DNMT1, DNMT3a, and DNMT3b in gastric cancer and their possible predictive relevance in future clinical practice.

MATERIALS AND METHODS

Patients and samples

From April 2000 to December 2010, 307 patients, including 233 men and 74 women with gastric cancer who underwent surgery at the First Hospital of Jilin University were collected. Finally, 227 men and 73 women patients were enrolled in the study. None of the patients received adjuvant chemotherapy or radiotherapy before the surgical treatment. All specimens obtained after surgery were collected. The pathological diagnosis of gastric cancer was made on the basis of morphologic and immunohistochemical findings by senior pathologists. Adjacent normal gastric epithelial samples were collected from 85 patients as comparison controls. Patients ranged in age from 32 to 87 years, with a median age of 63 years. The study protocol was approved by the Ethics Committee of the First Hospital of Jilin University. Written informed consent was obtained from all of the patients.

Immunohistochemistry

The 4 μm thickness sections from the tissue block were chosen for immunohistochemical staining. The sections were deparaffinized and stained using a streptavidin-biotin immunoperoxidase technique. Briefly, the tissue sections were incubated overnight at 4 °C with primary anti-human DNMT1 polyclonal antibody (1:200 diluted, sc-20701, Santa Cruz, United States), DNMT3a polyclonal antibody (1:200 diluted, sc-20703, Santa Cruz), and DNMT3b polyclonal antibody (1:200 diluted, sc-20704, Santa Cruz), respectively. Signals were visualized with 3,3-Diaminobenzidine (DAB) and the slides were counterstained with hematoxylin. As negative controls, the slides were treated with the isotype IgGs to replace primary antibodies, respectively. The stained slides were evaluated by two independent pathologists (MSJ and YPW), who were blinded from clinical data and outcome. The widely accepted HSCORE system was used to assess staining intensity and percentages of cells stained with a specific magnitude of intensity. Briefly, the HSCORE was calculated by the following equation: HSCORE = Σ Pi(i) (i = 0, 1, 2, 3, Pi = 0%-100%), where i means the intensity of staining, i.e. no staining = 0, weak staining = 1, moderate staining = 2, and strong staining = 3; Pi repre-
Table 1  Expression of DNA methyltransferases 1, 3a and 3b between different groups

|                | Gastric cancer (n = 85) | Control (n = 85) | H. pylori (+) cancer (n = 67) | H. pylori (-) cancer (n = 34) | P value |
|----------------|------------------------|----------------|-------------------------------|-------------------------------|---------|
| DNMT1          | 10 (0-40)              | 0 (0-5)        | 20 (0-120)                    | 50 (5-110)                    | 0.302   |
| DNMT3a         | 80 (5-150)             | 0 (0-0)        | 180 (40-210)                  | 155 (8-240)                   | 0.859   |
| DNMT3b         | 180 (140-240)          | 90 (0-240)     | 180 (120-240)                 | 160 (98-210)                  | 0.179   |

HSCOREs were represented with median (quartile range). H. pylori: Helicobacter pylori; DNMT: DNA methyltransferase.

RESULTS

Expression of DNMTs in gastric cancer and normal epithelial cells

In gastric cancer, expression of DNMTs was mainly seen in the nucleus. Weak staining was also observed in the cytoplasm (Figure 1). All negative controls demonstrated negligible background staining. Among the 85 paired samples, DNMT1, DNMT3a and DNMT3b positive staining were found in 51/85, 52/85, and 80/85 in gastric cancer samples, respectively. They were significantly higher compared to those in the paired control samples (60.0% vs 37.6%, 61.2% vs 4.7%, and 94.1% vs 71.8%, P < 0.001) (Table 1).

H. pylori infection did not induce expression of DNMTs

H. pylori infection was tested in the 101 gastric cancer patients, and the positive rate of H. pylori infection was 66.3% (67/101). However, the analysis results showed no correlations between H. pylori infection and the expression levels of DNMT1, DNMT3a, and DNMT3b (P = 0.302, 0.859, and 0.179, respectively) (Table 1).

Correlation of DNMT expression with clinicopathologic parameters

Expression of DNMT1 was significantly associated with lymph node metastasis of gastric cancer (P = 0.001). Meanwhile, there were significant higher HSCOREs of DNMT3a staining in patients with lymph-vascular invasion than those without infiltration (P = 0.02). There were significant higher HSCOREs of DNMT3b in patients with poor differentiation compared to those with well and moderate differentiation (P < 0.001). DNMT expression according to age, sex, and tumor differentiation, depth of invasion, lymph node metastasis, and distant metastasis and TNM stage were also analyzed and summarized in Table 2.

DNMT3a expression is associated with poor survival

Follow-up information was available for all 300 patients, covering periods ranging from 3 to 140 mo (median 41 mo). No patient died of postoperative complications within 30 d of the beginning of the study period, and 120 (40.0%) patients had died during the follow-up. The overall survival time was significantly longer in the DNMT3a negative group than in the DNMT3a positive group (Figure 2B, Log-rank test, P = 0.032). However, expression of DNMT1 and DNMT3b was not significantly associated with survival (Figure 2A, 2C, P = 0.289, P = 0.347). The analysis also showed that TNM stage was significantly related to postoperative survival (P < 0.001).

Expression of DNMT3a is an independent prognostic marker

After adjusted for gender, age, TNM stage and lymph-vascular invasion, patients with DNMT3a positive expression showed a significant difference in risk of gastric cancer-related deaths compared to those who were negative for DNMT3a expression (OR = 1.60, 95%CI: 1.06-2.40, P = 0.025). In multivariate analyses, DNMT3a expression and TNM stage were independent prognostic factors of poor patient survival in gastric cancer. However, expression of DNMT1 and DNMT3b was not associated with prognosis of gastric cancer (P = 0.542,
DISCUSSION

A great number of genes with promoter methylation have been observed in gastric cancer. It is believed that increased expression of DNMTs may contribute to the excessive methylation. Recently, the DNMT activity of DNMT1, DNMT3a and DNMT3b has been confirmed. Several studies have focused on the clinicopathology and DNMT expression in human cancers. However, the prognostic significance of DNMT expression in gastric carcinoma has not been explored thoroughly.

DNMT1 is a major and best known DNMT, and it can transfers methyl groups from S-adenosylmethionine to cytosines. DNMT1 can interact with the DNMT1-associated protein 1, histone deacetylases 1 and 2 and Rb and repress gene transcription. Etoh et al. reported that DNMT1 plays an important role in the development of poorly differentiated gastric cancer, by inducing frequent DNA hypermethylation in multiple CpG islands. Kanai et al. reported DNMT1 protein expression and DNA methylation status of CpG islands in tumor-related genes during multistage carcinogenesis of the pancreas. They found that the average number of methylated genes in ductal carcinomas was significantly correlated with DNMT1 protein expression level ($P < 0.0093$). Mutze et al. analyzed DNMT1/3b expression immunohistochemically in 127 pre-therapeutic biopsies from neoadjuvant-treated gastric cancer patients. They found that DNMT1 was a predictive biomarker and potential target for chemotherapy in gastric cancer. In the present study, DNMT1 expression was significantly higher in cancer.
tissue compared with the paired non-cancer mucosa. However, there was no difference between prognosis and expression levels. The results consist with a previous study[12]. Thus, it can be speculated that DNMT1 protein expression may play an essential effect in the carcinogenesis, but it was not associated with prognosis of gastric cancer.

DNMT3a and DNMT3b do show de novo DNA methylation activity in vitro, and they are responsible for the creation of methylation patterns at an early stage of embryogenesis. In 2008, Ding et al[12] investigated 38 gastric cancer patients and they failed to find the positive association between immunoreactivity of DNMT1, DNMT3a and DNMT3b in 54 gastric cancer patients, and they reported that co-expression of DNMT1 and DNMT3a was significantly associated with lymph node metastasis. In this study, we only found expression of DNMT1 was significantly associated with lymph node metastasis. However, DNMT3a expression was significantly associated with lymph-vascular invasion. Furthermore, in the follow-up study, expression of DNMT3a was detected as an independent prognostic marker. The inconsistent results from above studies may be caused by different study subjects and environmental backgrounds.

*H. pylori* infection is believed to be involved in gastric carcinogenesis, and has also been reported to strongly promote regional DNA hypermethylation. However, in the current study, *H. pylori* infection did not induce expression of DNMTs. Recently, Huang et al[28] found that there were no significant alterations in the total DNMT activities in mice challenged with *H. pylori*. Thus, although *H. pylori* infection alters DNA methylation, it did not induce expression of DNMTs. The mechanism is still unclear and warrants further investigation[26].

Most epigenetic modifications are post-transcrip-

### Table 2 DNA methyltransferases 1, 3a and 3b expression (HSCORE) in gastric cancer according to clinicopathologic parameters

|                  | DNMT1 Median (quantile range) | *P* value | DNMT3a Median (quantile range) | *P* value | DNMT3b Median (quantile range) | *P* value |
|------------------|--------------------------------|-----------|--------------------------------|-----------|--------------------------------|-----------|
| Gender           |                                |           |                                |           |                                |           |
| Male (n = 227)   | 10 (0-60)                      | 0.283     | 90 (0-180)                     | 0.629     | 210 (120-240)                  | 0.011     |
| Female (n = 73)  | 20 (0-90)                      |           | 80 (3-180)                     |           | 150 (95-240)                  |           |
| Age (yr)         |                                |           |                                |           |                                |           |
| ≤ 60 (n = 128)   | 10 (0-80)                      | 0.839     | 60 (1-180)                     | 0.101     | 160 (90-240)                  | 0.003     |
| > 60 (n = 172)   | 10 (0-60)                      |           | 100 (1-210)                    |           | 210 (140-240)                 |           |
| Smoking          |                                |           |                                |           |                                |           |
| Yes (n = 107)    | 10 (0-70)                      | 0.502     | 120 (5-210)                    | 0.065     | 180 (120-240)                 | 0.722     |
| No (n = 193)     | 10 (0-80)                      |           | 60 (1-180)                     |           | 180 (120-240)                 |           |
| Drinking         |                                |           |                                |           |                                |           |
| Yes (n = 72)     | 10 (0-80)                      | 0.570     | 120 (40-233)                   | 0.003     | 195 (125-240)                 | 0.777     |
| No (n = 228)     | 10 (0-60)                      |           | 60 (1-180)                     |           | 180 (120-240)                 |           |
| TNM stage        |                                |           |                                |           |                                |           |
| I (n = 22)       | 45 (5-93)                      | 0.106     | 80 (0-180)                     | 0.905     | 135 (75-240)                  | 0.149     |
| II (n = 51)      | 20 (0-88)                      |           | 60 (5-180)                     |           | 160 (90-240)                  |           |
| III (n = 195)    | 10 (0-60)                      |           | 90 (5-180)                     |           | 210 (140-240)                 |           |
| IV (n = 32)      | 10 (0-35)                      |           | 90 (0-180)                     |           | 170(90-240)                   |           |
| Differentiation  |                                |           |                                |           |                                |           |
| Well + moderate  | 5 (0-60)                       | 0.051     | 100 (5-210)                    | 0.158     | 160 (100-240)                 | <0.001    |
| Poor (n = 181)   | 20 (0-80)                      |           | 80 (0-180)                     |           | 240 (150-270)                 |           |
| Lymph-vascular invasion | 10 (0-60) | 0.159     | 60 (0-180)                     | 0.020     | 180 (120-240)                 | 0.942     |
| Present (n = 162) | 10 (0-80)                     |           | 110 (10-210)                   |           | 180 (120-240)                 |           |
| Depth of invasion|                                |           |                                |           |                                |           |
| T1 (n = 8)       | 80 (13-130)                    | 0.090     | 130 (20-225)                   | 0.082     | 185 (128-240)                 | 0.424     |
| T2 (n = 32)      | 20 (0-98)                      |           | 60 (0-180)                     |           | 180 (50-240)                  |           |
| T3 (n = 223)     | 10 (0-70)                      |           | 90 (10-210)                    |           | 180 (120-240)                 |           |
| T4 (n = 31)      | 10 (0-20)                      |           | 40 (1-140)                     |           | 180 (120-240)                 |           |
| Lymph metastasis |                                |           |                                |           |                                |           |
| N0 (n = 65)      | 30 (0-88)                      | 0.001     | 60 (0-170)                     | 0.503     | 180 (100-240)                 | 0.166     |
| N1 (n = 92)      | 0 (0-28)                       |           | 60 (0-203)                     |           | 210 (140-240)                 |           |
| N2 (n = 78)      | 20 (0-93)                      |           | 110 (5-188)                    |           | 180 (120-240)                 |           |
| N3 (n = 65)      | 20 (0-70)                      |           | 90 (15-180)                    |           | 180 (145-255)                 |           |
| Distant metastasis |                                |           |                                |           |                                |           |
| Negative (n = 265) | 10 (0-80)                 | 0.938     | 80 (5-180)                     | 0.679     | 195 (120-240)                 | 0.285     |
| Positive (n = 35) | 10 (0-60)                   |           | 90 (0-210)                     |           | 160 (90-240)                  |           |
| Survival         |                                |           |                                |           |                                |           |
| Survival (n = 180) | 10 (0-80)                 | 0.656     | 60 (0-180)                     | 0.009     | 180 (120-240)                 | 0.441     |
| Death (n = 120)  | 10 (0-60)                      |           | 120 (13-210)                   |           | 210 (120-240)                 |           |

DNMT: DNA methyltransferase.
In conclusion, aberrant expression of DNMT3a plays a crucial role in gastric carcinogenesis. Expression of DNMT3a was associated with poor survival in gastric carcinoma. This suggested that DNMT3a is clinically useful for prediction of prognosis of gastric cancer, and could be useful as a therapeutic target. Since the function of DNMT3a in gastric carcinogenesis is still unclear, future research is needed\(^{28-32}\).

### Table 3 Multivariate analysis of predictors of overall survival in gastric cancer

| HR (95%CI) | P value* |
|------------|----------|
| **DNMT1 expression** |  |
| Negative (n = 180) | Reference | 0.542 |
| Positive (n = 120) | 0.89 (0.60-1.31) |  |
| **DNMT3a expression** |  |
| Negative (n = 106) | Reference | 0.025 |
| Positive (n = 194) | 1.60 (1.06-2.40) |  |
| **DNMT3b expression** |  |
| HSCORE ≤ 200 (n = 154) | Reference | 0.620 |
| HSCORE > 200 (n = 146) | 1.17 (0.63-2.18) |  |
| **Lymph-vascular invasion** |  |
| Absent (n = 138) | Reference | 0.130 |
| Present (n = 162) | 1.36 (0.91-2.05) |  |
| **TNM stage** |  |
| 0 (n = 22) | Reference | 0.289 |
| I (n = 51) | 1.78 (0.50-6.37) | 0.379 |
| II (n = 195) | 4.11 (1.30-13.05) | 0.016 |
| IV (n = 32) | 14.55 (4.38-48.31) | < 0.001 |

* Cox proportional hazards model, adjusted for gender, age, smoking, drinking, lymph-vascular invasion, and DNMT expression. DNMT: DNA methyltransferase.

### COMMENTS

**Background**

DNA methylation mediated by DNA methyltransferases (DNMTs) plays an important role in cancer. Few studies have investigated the relationship between expression of the DNMTs and prognosis in gastric cancer.

**Research frontiers**

Previous studies reported the clinical significance of the expression of DNMTs and their application as potential prognostic markers in gastric cancer.

**Innovations and breakthroughs**

In this study, DNMT3a expression was detected as a new independent prognostic marker in gastric cancer.

**Applications**

Expression of DNMT3a was associated with poor survival in gastric cancer. This suggested that DNMT3a is clinically useful for prediction of prognosis of gastric cancer, and it was considered as a potential therapeutic target.

**Terminology**

DNA methyltransferases 3a (DNMT3a) is an enzyme that catalyzes the transfer of methyl groups to specific CpG structures in DNA, a process called DNA methylation.

**Peer review**

Generally, the authors made a significant research of gastric cancer. With the basic study, the authors demonstrated that DNMT3a, which was a methyltransferase of DNA, was an independent factor of OS in patients with gastric cancer.

**REFERENCES**

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]
2 Sasaki M, Inoue M, Lin JT, Khor C, Yang HK, Ohtsu A. Gastric Cancer Working Group report. Jpn J Clin Oncol 2010; 40 Suppl 1: 128-137 [PMID: 20870917 DOI: 10.1093/jjco/hyp024]

3 Daniel FL, Cherubini K, Yurgel LS, de Figueiredo MA, Salum FG. The role of epigenetic transcription repression and DNA methyltransferases in cancer. Cancer 2011; 117: 677-687 [PMID: 20945317 DOI: 10.1002/cncr.25482]

4 Leclerc D, Lévesque N, Cao Y, Deng L, Wu Q, Powell J, Sapienza C, Rozen R. Genes with aberrant expression in murine preneoplastic intestine show epigenetic and expression changes in normal mucosa of colon cancer patients. Cancer Prev Res (Phila) 2013; 6: 1171-1181 [PMID: 24169962 DOI: 10.1186/1940-6207]

5 Previati M, Manfrini M, Galasso M, Zerbini C, Palatini J, Gasparini P, Volinia S. Next generation analysis of breast cancer genomes for precision medicine. Cancer Lett 2013; 339: l-7 [PMID: 23879964 DOI: 10.1016/j.canlet.2013.07.018]

6 Chen XY, He QY, Guo MZ. XAF1 is frequently methylated in human esophageal cancer. World J Gastroenterol 2012; 18: 2844-2849 [PMID: 22719195 DOI: 10.3748/wgj.v18.i22.2844]

7 Chik F, Szyf M. Effects of specific DNMT gene depletion on cancer cell transformation and breast cancer cell invasion; toward selective DNMT inhibitors. Carcinogenesis 2011; 32: 224-232 [PMID: 20980350 DOI: 10.1093/carcin/bgp221]

8 He S, Wang F, Yang X, Guo C, Wang X. Expression of DNMT1 and DNMT3a are regulated by GLI1 in human pancreatic cancer. PLoS One 2011; 6: e27684 [PMID: 22110720 DOI: 10.1371/journal.pone.0027684]

9 Calcagno DQ, Gigek CO, Chen ES, Burbano RR, Smith MD. A DNA and histone methylation in gastric carcinogenesis. World J Gastroenterol 2013; 19: 1182-1192 [PMID: 23882412 DOI: 10.3748/wgj.v19.i8.1182]

10 Psotaki V, Kalogerap C, Tsambouras N, Stephanou D, Tsiaras E, Seferiadis K, Kolios G. Promoter methylation status of hMLH1, MGMT, and CKN2A/p16 in colorectal adenomas. World J Gastroenterol 2010; 16: 3553-3560 [PMID: 20653064 DOI: 10.3748/wjg.v16.i28.3553]

11 Kim SJ, Zhao H, Hardikar S, Singh AK, Goodell MA, Chen T. DNA methylation in interleukin-1 receptor type 1 knockout (IL-1R1(-/-)) mice. J Exp Med 2011; 208: 2844-2849 [PMID: 22719195 DOI: 10.1038/jem.2011.578]

12 Ding WY, Wang JY, Chen XY, Peng VS. The expression and clinical significance of DNA methyltransferase proteins in human gastric cancer. Dig Dis Sci 2008; 53: 2083-2089 [PMID: 18253890 DOI: 10.1007/s10620-007-0145-2]

13 Yang J, Wei X, Wu Q, Xu Z, Gu D, Jin Y, Shen Y, Huang H, Fan H, Chen J. Clinical significance of the expression of DNA methyltransferase proteins in gastric cancer. Mol Med Rep 2011; 4: 1139-1143 [PMID: 21887466 DOI: 10.3802/mmrr.2011.578]

14 Jiang J, Jia Z, Cao D, Jin MS, Kong F, Su J, Cao X. Polymorphisms of the DNA methyltransferase 1 associated with reduced risks of Helicobacter pylori infection and increased risks of gastric atrophy. PLoS One 2012; 7: e46058 [PMID: 23049933 DOI: 10.1371/journal.pone.0046058]

15 Cao XY, Jia ZF, Jin MS, Cao DH, Kong F, Su J, Jiang J. Serum pepsinogen II is a better diagnostic marker in gastric cancer. World J Gastroenterol 2012; 18: 7357-7361 [PMID: 23362145 DOI: 10.3748/wjg.v18.i48.7357]

16 Kim H, Park J, Jung Y, Song SH, Han SW, Oh DY, Im SA, Bang YJ, Kim TY. DNA methyltransferase 3-like affects promoter methylation of thymine DNA glycosylase independently of DNMT1 and DNMT3B in cancer cells. Int J Oncol 2010; 36: 1563-1572 [PMID: 20425871 DOI: 10.3802/ijo_0000643]

17 Esteller M. Cancer epigenomics: DNA methylomes and histone-modification maps. Nat Rev Genet 2007; 8: 286-298 [PMID: 17539880 DOI: 10.1038/nrg2005]

18 Arai E, Nakagawa T, Wakai-Ushijima S, Fujimoto H, Kawai N. DNA methyltransferase 3B expression is associated with poor outcome of stage 1 testicular seminoma. Histopathology 2012; 60: E12-E18 [PMID: 22394436 DOI: 10.1111/j.1365-2559.2012.04174.x]

19 Qu Y, Mu G, Wu Y, Dai X, Zhou F, Xu X, Wang Y, Wei F. Overexpression of DNA methyltransferases 1, 3a, and 3b significantly correlates with retinoblastoma tumorigenesis. Ann J Clin Pathol 2010; 134: 826-834 [PMID: 20959668 DOI: 10.1309/ACJTHQG69FDPDW]

20 Rahman MM, Qian ZR, Wang EL, Yoshimoto K, Nakagawa T, Wakai-Ushijima S, Fujimoto H, Kamiya M, Kitano S, Hirohashi S. Increased DNA methyltransferase 1 (DNMT1) protein expression correlates significantly with poorer tumor differentiation and frequent DNA hypermethylation of multiple CpG islands in gastric cancers. Am J Pathol 2004; 164: 689-699 [PMID: 14742272]

21 Kanai Y, Hiroshi S. Alterations of DNA methylation associated with abnormalities of DNA methyltransferases in humans during transition from a precancerous to a malignant state. Carcinogenesis 2007; 28: 2434-2442 [PMID: 17989243 DOI: 10.1093/carcin/bgm206]

22 Mutke K, Langer R, Schumacher F, Becker K, Ott K, Novotny A, Hafelmeier A, Höfler H, Keller G. DNA methyltransferase 1 as a predictive biomarker and potential therapeutic target for chemotherapy in gastric cancer. Eur J Cancer 2011; 47: 1817-1825 [PMID: 21458988 DOI: 10.1016/j.ejca.2011.02.024]

23 Huang FY, Chan AO, Lo RC, Rashid A, Wong DK, Cho CH, Lai CL, Yuen MF. Characterization of interleukin-1β in Helicobacter pylori-induced gastric inflammation and DNA methylation in interleukin-1 receptor type 1 knockout (IL-1R1(-/-)) mice. Eur J Cancer Prev 2013; 22: 2760-2770 [PMID: 23646095 DOI: 10.1097/CEJ.0b013e32836303f1]

24 Huang FY, Chan AO, Rashid A, Wong DK, Cho CH, Yuen MF. Helicobacter pylori induces promoter methylation of E-cadherin via interleukin-1β activation of nitric oxide production in gastric cancer cells. Cancer 2012; 118: 4969-4980 [PMID: 22415887 DOI: 10.1002/cncr.27519]

25 Niwa T, Toyota T, Tsukamoto T, Morii A, Tatematsu M, Ushijima T, Prealignment of Helicobacter pylori-induced gastric cancers in gerbils by a DNA demethylating agent. Cancer Prev Res (Phila) 2013; 6: 263-270 [PMID: 23559452 DOI: 10.1158/1940-6207.CAPR-12-0369]

26 Tang H, Deng M, Tang Y, Xie X, Guo J, Kong Y, Ye F, Su Q, Xie X. miR-200b and miR-200c as prognostic factors and mediators of gastric cancer cell progression. Clin Cancer Res 2013; 19: 5602-5612 [PMID: 23995857 DOI: 10.1158/1078-0432.CCR-13-3381]

27 Zhang JJ, Zhu Y, Zhu Y, Wu JL, Liang WB, Zhu R, Xu ZX, Du Q, Miao Y. Association of increased DNA methyltransferase expression with carcinogenesis and poor prognosis in pancreatic ductal adenocarcinoma. Clin Transl Oncol 2012; 14: 116-124 [PMID: 22301400 DOI: 10.1007/s12014-012-0770-x]

28 Qu Y, Deng S, Hou P. Gene methylation in gastric cancer. Clin Chim Acta 2013; 424: 53-65 [PMID: 23669186 DOI: 10.1016/j.cca.2013.05.047]
Cao XY et al. DNMT3a expression in gastric cancer

10.1016/j.cca.2013.05.002

Chihara Y, Kanai Y, Fujimoto H, Sugano K, Kawashima K, Liang G, Jones PA, Fujimoto K, Kuniyasu H, Hirao Y. Diagnostic markers of urothelial cancer based on DNA methylation analysis. BMC Cancer 2013; 13: 275 [PMID: 23735005 DOI: 10.1186/1471-2407-13-275]

He M, Fan J, Jiang R, Tang WX, Wang ZW. Expression of DNMTs and genomic DNA methylation in gastric signet ring cell carcinoma. Mol Med Rep 2013; 8: 942-948 [PMID: 23820855 DOI: 10.3892/mmr.2013.1566]

P- Reviewer: Liang H  S- Editor: Ma YJ  L- Editor: Wang TQ  E- Editor: Liu XM
