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Expression and Prognostic Significance of O^6-Methylguanine-DNA Methyltransferase in Hepatocellular, Gastric, and Breast Cancers

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Background: O^6-Methylguanine-DNA methyltransferase (MGMT) is an enzyme that repairs O^6-methylguanine, a promutagenic DNA base damaged by endogenous and environmental alkylating agents. There are few reports that describe whether or not abnormal MGMT expression correlates with the prognosis in human solid cancers.

Methods: The expression of MGMT was immunohistochemically evaluated in 60, 62, 105, and 46 paraffin-embedded samples from patients with curatively resected hepatocellular, gastric, colorectal, and breast cancers, respectively.

Results: The expression of MGMT was a positive predictive factor for overall survival in hepatocellular ($P = .005$) and gastric cancers ($P < .001$) and for relapse-free survival in breast cancers ($P < .001$). MGMT-positive gastric tumors (n = 42) were correlated with the absence of serosal invasion ($P = .045$), lymph node metastasis ($P = .006$), intestinal type ($P = .018$), and low pathological tumor, node, metastasis stage ($P < .001$). All breast tumors that recurred locally after operation were MGMT negative ($P = .004$). The clinicopathologic characteristics of colorectal cancers with respect to MGMT expression did not significantly differ.

Conclusions: The expression of MGMT is a predictive prognostic marker in patients with hepatocellular, gastric, and breast cancers. These findings may help to establish therapeutic strategies for patients with these types of solid cancer.

Key Words: O^6-methylguanine-DNA methyltransferase (MGMT)—Hepatocellular carcinoma—Gastric cancer—Colorectal cancer—Breast cancer—Prognosis.

Ubiquitous and environmental alkylating agents such as *N*-nitroso compounds are principally metabolized and activated in hepatocytes.[1] Because endogenous alkylating compounds are released into bile and the digestive tract, epithelial cells in the biliary and gastrointestinal tract are always exposed to activated alkylating agents.[2] Alkylating agents cause gene mutations or cell death in vitro[3] and carcinogenesis or apoptosis in vivo.[4,5] These biological effects are induced by the promutagenic base, O^6-methylguanine, which is produced by the alkylating agents.[6] O^6-Methylguanine preferentially mispairs with thymine instead of cytosine during DNA replication, leading to a G:C/A:T transition mutation.[7] Humans possess O^6-methylguanine-DNA methyltransferase (MGMT), which repairs O^6-methylguanine to prevent such mispairing.[8] Abnormal MGMT expression causes O^6-methylguanine to accumulate in cellular DNA,[9] and this could result in activation of oncogenes or inactivation of tumor suppressor genes, contributing to carcinogenesis or tumor progression.[10–12] Recent findings from animal models and in

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vitro studies demonstrate that a deficiency in O^6-methylguanine repair seems to be one major determinant of susceptibility to carcinogenesis by alkylating agents.[13,14] The carcinogenic mechanism induced by disrupting the *MGMT* gene was convincingly demonstrated by using the transgenic or knockout mouse model.[4,5,15,16] If activation of oncogenes or inactivation of tumor suppresser genes arises because of abnormal MGMT expression in humans, alterations in such cancer-related genes accumulate. However, reports describing solid cancers and whether or not abnormal expression of MGMT correlates with the tumor grade or the prognosis are scarce.

Zaidi et al.[17] reported that MGMT immunohistochemical staining correlates with protein quantity and activity. Thus, immunohistochemical analysis can determine both the expression and distribution of MGMT protein. This study investigates the relationship between negative expression of MGMT determined by immunohistochemistry and clinicopathologic features, including prognosis, to clarify whether or not abnormal MGMT expression participates in the carcinogenesis and tumor progression of hepatocellular, gastric, colorectal, and breast cancers.

MATERIALS AND METHODS

Patients and Tumor Specimens

Patients with primary hepatocellular carcinoma (HCC), gastric cancer, colorectal cancer, and breast cancer admitted to the Department of Surgery, Saga Medical School Hospital, were considered for inclusion in this study. This study included consecutive series of 60, 62, 105, and 46 patients with primary hepatocellular, gastric, colorectal, and breast cancer from 1991 to 1998, 1991 to 1992, 1990 to 1992, and 1982 to 1997, respectively. We selected patients with HCC who had simultaneously conserved fresh-frozen samples to investigate genomic analysis. For breast cancer patients, those without preoperative systemic chemotherapy were selected. The series of patients with gastric and colorectal cancer were completely consecutive. Follow-up data for retrospective analyses were obtained by reviewing patient records and by contacting patients' families and physicians. The mean follow-up period of patients with hepatocellular, gastric, colorectal, and breast cancer was 22.0 months (range, 5 to 80), 56.0 months (range, 10 to 82), 56.4 months (range, 10 to 90), and 63.8 months (range, 5 to 168) months, respectively. Representative formalin-fixed, paraffin-embedded tumor specimens from patients with each cancer who underwent curative resection were selected for this study. The clinical and pathologic features of patients with each type of cancer were classified with the *UICC TNM Classification of Malignant Tumors.*[18]

Anti-MGMT Antibody

Because the commercialized anti-human MGMT antibody was nonexistent when this study was started, we used the MGMT custom-made antibody. Polyclonal rabbit antibodies against human MGMT protein were prepared by using TrpE fusion protein, as described.[19] *Escherichia coli* BL21 (DE3) carrying pET3d:TrpE-hMGMT-1, which encodes the TrpE polypeptide, fused to a region of MGMT (residues 1 to 45) at the C terminus was used to produce each fusion protein,[20] and polyclonal antibodies against the fusion protein were raised in rabbits. The serum was initially applied to a TrpE-hMGMT-1-coupled column, and their bound materials were eluted at pH 2.3 and dialyzed against 10 mM of Tris-HCl (pH 7.4) and 150 mM of NaCl. To increase antibody specificity, the eluted fraction was applied to an affinity column with TrpE-mMGMT-1, in which a corresponding region of mouse MGMT (residues 1 to 58) was fused to TrpE[21] as a ligand, and then the bound fraction was eluted and dialyzed. This fraction was used as anti-MGMT antibody.

Immunohistochemical Staining for MGMT

Sections (5 μm) were deparaffinized in xylene and dehydrated. Antigen was retrieved by microwaving the

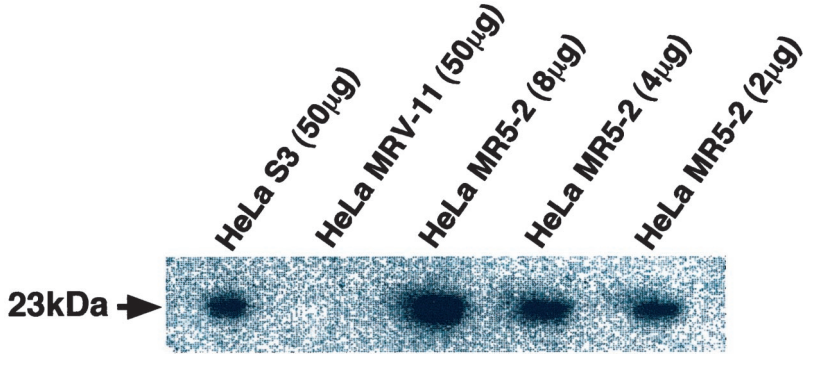

FIG. 1. The specificity of O^6-methylguanine-DNA methyltransferase (MGMT) antibody was determined by Western blot analysis by using cell-line lysate. HeLa S3 and HeLa MRV-11 were MGMT-positive and MGMT-negative cell lines, respectively. HeLa MR5-2 was an MGMT overexpressor. The band corresponded to MGMT protein (23 kDa). Semiquantitative analysis is shown. The amount of protein (μg) on loading is in parentheses.

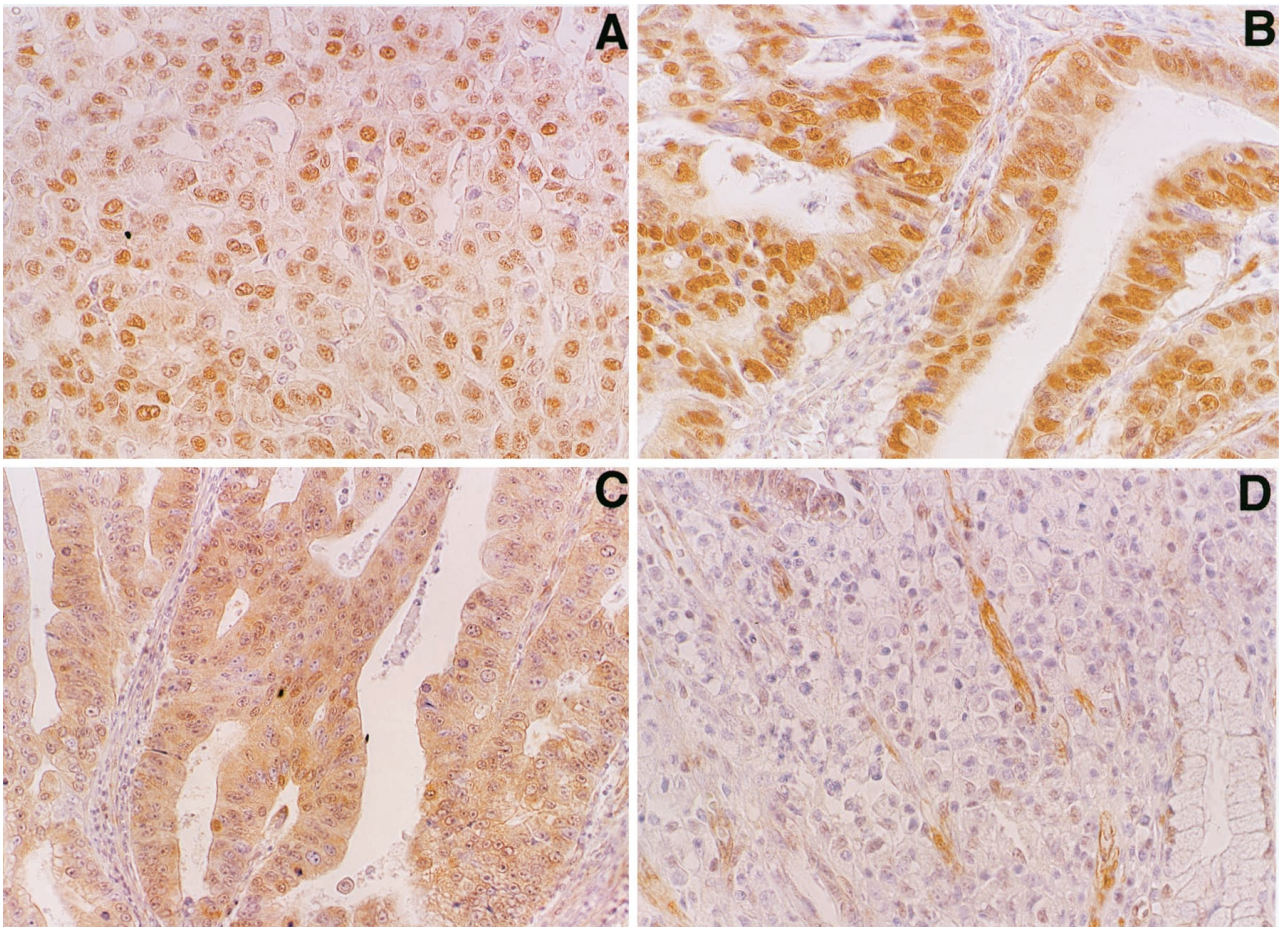

FIG. 2. O^6-methylguanine-DNA methyltransferase (MGMT) immunohistochemical staining of hepatocellular carcinoma (HCC) and gastric cancer (magnification: A, B, D, ×200; C, ×100). **(A)** Representative MGMT-positive HCC; **(B, C)** MGMT-positive gastric cancer. Gastric cancer with MGMT proteins stained in nuclei **(B)** or cytoplasm **(C)** were defined as MGMT positive. No signal for protein was detected in MGMT-negative gastric cancer **(D)**. Interstitial fibroblast was shown as an internal positive control **(D)**.

samples three times for 5 minutes in 10 mM of sodium citrate buffer (pH 6.5). Endogenous peroxidase activities were blocked by immersing the slides in methanol containing 3% hydrogen peroxide for 10 minutes. The slides were then incubated with 10% normal goat serum (Nichirei Co., Tokyo, Japan) for 30 minutes to reduce background staining, followed by anti-human MGMT antibodies (1/200) at 4°C overnight. Negative control sections were incubated with normal rabbit serum instead of the antibodies. The slides were then exposed to EnVision+™ polymer reagent (Dako Corporation, Carpinteria, CA), and goat anti-rabbit immunoglobulins were conjugated with peroxidase-labeled dextran polymer at room temperature for 30 minutes. The slides were washed in phosphate-buffered saline twice and developed by using a Histofine DAB™ substrate kit (Nichirei) according to the manufacturer's instructions at room temperature for 8 minutes. Nuclei were counterstained with hematoxylin. Normal epithelia, interstitial fibroblast, vascular smooth muscle, and smooth muscle layers within the sections were used as internal positive controls. Two pathologists without knowledge of clinicopathologic features and clinical outcome of the patients assessed the status of MGMT expression as positive or negative. The sample was considered positive when immunoreactivity was detected in >10% of the cells in nuclei, cytoplasm, or both.[22,23]

Statistical Analysis

The clinicopathologic characteristics were compared with MGMT-positive and -negative groups, and the significance of associations was determined with the Mann-Whitney *U*-test or Student's *t*-test for continuous data and the χ^2 test for categorical data. The survival data were used to generate Kaplan-Meier curves that were compared on the basis of MGMT status by using the

TABLE 1. *Correlation of MGMT expression with clinicopathologic features in hepatocellular carcinoma*

Patient and tumor characteristics	No. patients	MGMT Positive (%)	MGMT Negative (%)
Total tumors	60	30 (50.0)	30 (50.0)
Age, y (mean ± SD)	63 ± 5	63 ± 4	63 ± 6
Sex (male:female)	46:14	21:9	25:5
Viral infection			
HBV(+)	11	4 (36.4)	7 (63.6)
HCV(+)	43	22 (51.2)	21 (48.8)
HBV(+) and HCV(+)	2	0 (0.0)	2 (100)
None	4	4 (100)	0 (0.0)
Liver cirrhosis			
Absent	5	3 (60.0)	2 (40.0)
Present	55	27 (49.1)	28 (50.9)
Tumor diameter, cm (mean ± SD)	4.1 ± 1.5	4.1 ± 1.4	4.1 ± 1.6
Grade of differentiation[a]			
G1	17	10 (58.8)	7 (41.2)
G2	29	13 (44.8)	16 (55.2)
G3	6	2 (33.3)	4 (66.7)
GX	8	5 (62.5)	3 (37.5)
Portal invasion			
Absent	49	28 (57.1)	21 (42.9)
Present	11	2 (18.2)	9 (81.8)
Intrahepatic metastasis			
Absent	35	19 (54.3)	16 (45.7)
Present	25	11 (44.0)	14 (56.0)
pTNM stage[a]			
I	4	3 (75.0)	1 (25.0)
II	28	17 (60.7)	11 (39.3)
IIIA	20	7 (35.0)	13 (65.0)
IVA	8	3 (37.5)	5 (62.5)

HBV, hepatitis B virus; HCV, hepatitis C virus; pTNM, pathological tumor, node, metastasis; MGMT, O^6-methylguanine-DNA methyltransferase.

[a] Grade of differentiation and stage were established according to Ref. 18.

log-rank test. Statistical significance was judged as $P < .05$. Multivariate analysis with the Cox proportional hazards regression model was performed by using statistical analysis software (JMP Macintosh™ version; SAS Institute, Cary, NC).

RESULTS

Determination of Anti-MGMT Antibody

We determined the specificity of this antibody by Western blot analysis by using cell-line lysate. HeLa S3 is an MGMT-proficient (Mer^+) cell line[24,25] of cervical carcinoma. HeLa MR is an MGMT-deficient (Mer^-) cell line,[24,25] and HeLa MRV-11 is transfected vector only. HeLa MR5-2 is an MGMT overexpressor that is transfected with human *MGMT* expression vector. The band corresponded to MGMT protein (23 kDa; Fig. 1). Semiquantitative analysis is shown in Fig. 1.

Expression of MGMT

MGMT proteins were expressed intensely and uniformly in the nuclei and cytoplasm of cell lines and were detected in normal epithelia, interstitial fibroblasts, vascular smooth muscle, and the smooth muscle layer (data not shown). Tumors positive for MGMT homogeneously expressed the protein in the nuclei of HCC (Fig. 2A), gastric cancer (Fig. 2B), colorectal cancer, and breast cancer (data not shown) cells. Several cancers in which signals for the protein were detected in the cytoplasm were classified as MGMT positive (Fig. 2C, gastric cancer). Tumors without signals for the protein in the nuclei and cytoplasm were defined as MGMT negative (Fig. 2D, gastric cancer). Interstitial fibroblast was shown as an internal positive control in Fig. 2D. MGMT positivity was identified in 50.0% (30 patients) of 60 HCCs, 67.7% (42 patients) of 62 gastric cancers, 33.3% (35 patients) of 105 colorectal cancers, and 54.3% (25 patients) of 46 breast cancers.

Correlation Between MGMT Expression Status and Clinicopathologic Features

HCC

No significant correlation was found between MGMT expression status and age, sex, viral infection, liver cirrhosis, tumor diameter, grade of differentiation, portal invasion or intrahepatic metastasis, or pathologic tumor, node, metastasis stage (Table 1).

Gastric Cancer

Serosal invasion, lymph node metastasis, histologic type, and pathologic tumor, node, metastasis stage of the gastric cancers were associated with MGMT expression status, with a significant difference ($P = .045$, $P = .006$, $P = .018$, and $P < .001$, respectively; Table 2). MGMT-negative tumors invaded deeper into the stomach wall, had a higher ratio of the present lymph node metastasis and diffuse type, and were classified at a higher pathologic tumor, node, metastasis stage than MGMT-positive tumors. MGMT expression and the other clinicopathologic features analyzed in the study did not significantly correlate.

Colorectal Cancer

The MGMT expression status and age, sex, tumor size, tumor location, lymphatic invasion, venous invasion, serosal invasion, lymph node metastasis, grade of differentiation, and pathologic tumor, node, metastasis stage of colorectal cancer did not significantly correlate (Table 3).

Breast Cancer

All of the breast cancers were pathologically diagnosed as invasive ductal carcinoma. Because only two

TABLE 2. *Correlation of MGMT expression with clinicopathologic features in gastric cancer*

Patient and tumor characteristics	No. patients	MGMT		P value[a]
		Positive (%)	Negative (%)	
Total tumors	62	42 (67.7)	20 (32.3)	
Age, y (mean ± SD)	65 ± 6	66 ± 3	65 ± 5	NS
Sex (male:female)	46:16	32:10	14:6	NS
Tumor diameter, cm (mean ± SD)	4.5 ± 1.6	4.4 ± 1.8	4.6 ± 1.5	NS
Tumor location				NS
Upper	10	6 (60.0)	4 (40.0)	
Middle	22	13 (59.1)	9 (40.9)	
Lower	30	23 (76.7)	7 (23.3)	
Serosal invasion				.045
Absent	47	35 (74.5)	12 (25.5)	
Present	15	7 (46.7)	8 (53.3)	
Lymph node metastasis				.006
Absent	45	35 (77.8)	10 (22.2)	
Present	17	7 (41.2)	10 (58.8)	
Grade of differentiation[b]				NS
G1	17	14 (82.4)	3 (17.6)	
G2	21	16 (76.2)	5 (23.8)	
G3	16	9 (56.3)	7 (43.7)	
GX	8	3 (37.5)	5 (62.5)	
Histological type				.018
Intestinal	38	30 (78.9)	8 (21.1)	
Diffuse	24	12 (50.0)	12 (50.0)	
pTNM stage[b]				<.001
IA	28	27 (96.4)	1 (3.6)	
II	19	10 (52.6)	9 (47.4)	
IIIA	11	4 (36.4)	7 (63.6)	
IIIB	4	1 (25.0)	3 (75.0)	
IV	0	0 (0.0)	0 (0.0)	

NS, not statistically significant; MGMT, O^6-methylguanine-DNA methyltransferase; pTNM, pathological tumor, node, metastasis.
[a] *P* value calculated by χ^2 tests and Mann-Whitney *U*-tests for comparison of MGMT-positive and -negative groups.
[b] Grade of differentiation and stage were established according to Ref. 18.

patients with breast cancer died, we analyzed not overall survival but relapse-free survival rates. Local recurrence of MGMT-negative tumors was frequent, with a significant difference ($P = .004$; Table 4). The MGMT expression status and the other clinicopathologic characteristics did not significantly correlate.

Univariate Analysis of Survival

HCC

The overall 5-year survival rate for patients with MGMT-positive tumors was 89.1%, compared with 46.9% for those with MGMT-negative tumors ($P = .005$; Fig. 3A).

Gastric Cancer

The overall 5-year survival rates for patients with MGMT-positive and -negative tumors were 88.0% and 35.0%, respectively ($P < .001$; Fig. 3B).

Colorectal Cancer

The overall 5-year survival rates for patients with MGMT-positive and -negative tumors were 82.9% and 76.1%, respectively, with no significant difference ($P = .6521$; Fig. 3C).

Breast Cancer

The 10-year relapse-free survival rates for patients with MGMT-positive and -negative tumors were 100.0% and 35.7%, respectively ($P < .001$; Fig. 3D).

Multivariate Analysis of Survival

To determine the variables affecting the survival of gastric cancer patients, five variables correlated in univariate analysis (serosal invasion; lymph node metastasis; histologic type; pathological tumor, node, metastasis stage; and MGMT status) were analyzed by using the Cox proportional hazards regression model. Analysis showed pathological tumor, node, metastasis stage ($P = .0034$) and lymph node metastasis ($P = .0196$) to be significant variables to independently predict postoperative survival (Table 5). Among HCC patients, MGMT status (and likewise, pathological tumor, node, metastasis stage) was an independent prognostic factor (data not shown).

TABLE 3. *Correlation of MGMT expression with clinicopathologic features in colorectal cancer*

Patient and tumor characteristics	No. patients	MGMT Positive (%)	MGMT Negative (%)
Total tumors	105	35 (33.3)	70 (66.7)
Age, y (mean ± SD)	68 ± 7	67 ± 6	69 ± 8
Sex (male:female)	64:41	22:13	42:28
Tumor diameter, cm (mean ± SD)	6.6 ± 3.0	6.5 ± 2.6	6.4 ± 2.3
Tumor location			
Colon	65	20 (30.8)	45 (69.2)
Rectum	40	15 (37.5)	25 (62.5)
Lymphatic invasion			
Absent	45	17 (37.8)	28 (62.2)
Present	60	18 (30.0)	42 (70.0)
Venous invasion			
Absent	18	7 (38.9)	11 (61.1)
Present	87	28 (32.2)	59 (67.8)
Serosal invasion			
Absent	81	25 (30.9)	56 (69.1)
Present	24	10 (41.7)	14 (58.3)
Lymph node metastasis			
Absent	68	21 (30.9)	47 (69.1)
Present	37	14 (37.8)	23 (62.2)
Grade of differentiation[a]			
G1	76	24 (31.6)	52 (68.4)
G2	25	9 (36.0)	16 (64.0)
G3	0	0 (0.0)	0 (0.0)
GX	4	2 (50.0)	2 (50.0)
pTNM stage[a]			
I	25	8 (32.0)	17 (68.0)
II	37	11 (29.7)	26 (70.3)
III	35	13 (37.1)	22 (62.9)
IV	8	3 (37.5)	5 (62.5)

MGMT, O^6-methylguanine-DNA methyltransferase; pTNM, pathological tumor, node, metastasis.

[a] Grade of differentiation and stage were established according to Ref. 18.

DISCUSSION

The expression of MGMT protein has been studied. Kokkinakis et al.[26] reported that the MGMT protein expression level in pancreatic cancer was correlated with malignant potential. They showed that tumors that expressed high levels of MGMT protein had lower grade differentiation, more advanced stage, and a poorer prognosis than the tumors with low expression.[26] This study found that tumors with negative expression had a poorer prognosis than those with positive expression in hepatocellular, gastric, and breast cancer. This discrepancy may be due to the different numbers of patients and the study scale. Because Kokkinakis et al. studied only 12 patients with invasive ductal adenocarcinoma and did not include early-stage disease, statistical significance was not demonstrated. Ishibashi et al.[24] suggested that the intracellular distribution of MGMT differs among tumor types and in some cancer cell lines. The expression pattern of pancreatic cancer might be different from that of the cancers in our study.

This study of patients with HCC found that negative MGMT expression was significantly correlated with poor prognosis. MGMT status did not significantly differ with respect to clinicopathologic features, and multivariate analysis of survival showed that it is an independent predictive prognostic factor (data not shown). It is unclear why MGMT expression could be an independent prognostic marker. It is speculated that MGMT status might be correlated with potential liver function.[27] Major and Collier[27] demonstrated that the mean value of MGMT activities determined in the viral cirrhotic liver is much lower than that of normal tissue. However, in our study MGMT status did not differ between the presence and absence of viral infection or liver cirrhosis.

Negative MGMT expression was significantly correlated with tumor progression and a poor prognosis of gastric cancer. Because MGMT-negative gastric cancers had progressive characteristics, such as advanced pathologic tumor, node, metastasis stage, it is obvious why patients with MGMT-negative gastric cancer had a poorer prognosis. Multivariate analysis of the Cox proportional hazards model showed that MGMT expression status was not an independent prognostic factor. Pathological tumor, node, metastasis stage was the strongest prognostic marker. Thus, abnormalities in genes related to cancerous invasion and metastasis, such as adhesion molecules, should be involved in MGMT dysfunction.

In a study of breast cancer, however, Wani and D'Ambrosio[28] reported that the tumor grade and metastatic potential of breast cancer were not correlated with negative expression of the messenger RNA for the *MGMT* gene. This study of patients with breast cancer showed that tumor grade or metastasis and negative MGMT expression did not significantly correlate, whereas local recurrence and the relapse-free survival rate correlated with negative expression. Activation or inactivation of unknown molecules that contributed to the local recurrence may be involved in negative MGMT expression.

Among cancers analyzed in this study, a significant correlation between negative MGMT expression and the prognosis of patients with colorectal cancers was not found. The significance of this enigma may be organ specific, but early-stage cancers were not included in this study of colorectal cancers. For an explanation for the differences in the results for each tumor type, consideration should be given to varia-

TABLE 4. *Correlation of MGMT expression with clinicopathologic features in breast cancer*

Patient and tumor characteristics	No. patients	MGMT		P value[a]
		Positive (%)	Negative (%)	
Total tumors	46	25 (54.3)	21 (45.7)	
Age (y)				NS
Range	33–78	40–78	33–75	
Median	52	53	51	
<45	18	10 (55.6)	8 (44.4)	
≥45	28	15 (53.6)	13 (46.4)	
Tumor diameter, cm (mean ± SD)	2.5 ± 1.2	2.4 ± 1.2	2.5 ± 1.0	NS
Lymph node metastasis				NS
Absent	26	13 (50.0)	13 (50.0)	
Present	20	12 (60.0)	8 (40.0)	
<5 nodes	10	6 (60.0)	4 (40.0)	
≥5 nodes	10	6 (60.0)	4 (40.0)	
Histological grade[b]				NS
I	6	5 (83.3)	1 (16.7)	
II	33	16 (48.5)	17 (51.5)	
III	7	4 (57.1)	3 (42.9)	
Local recurrence				.004
Absent	40	25 (62.5)	15 (37.5)	
Present	6	0 (0.0)	6 (100)	
Distant metastasis				NS
Absent	46	25 (54.3)	21 (45.7)	
Present	0	0 (0.0)	0 (0.0)	
pTNM stage[c]				NS
I	13	8 (61.5)	5 (38.5)	
IIA	21	11 (52.4)	10 (47.6)	
IIB	7	4 (57.1)	3 (42.9)	
IIIA	5	2 (40.0)	3 (60.0)	
IV	0	0 (0.0)	0 (0.0)	

NS, not statistically significant; MGMT, O^6-methylguanine-DNA methyltransferase; pTNM, pathological tumor, node, metastasis.
[a] *P* value calculated by χ^2 test and Mann-Whitney *U*-tests for comparison of MGMT-positive and -negative groups.
[b] Histological grading according to Bloom, Richardson, and Elston.
[c] Stage was established according to Ref. 18.

tions of point mutations for each malignancy. In gene mutations, a large percentage of pancreas[29,30] and, to a lesser extent, colorectal[31,32] cancers have K-*ras* mutations. K-*ras* and other mutations may occur early in colorectal cancers,[33] and therefore evaluating MGMT expression may not be prognostic when evaluating more advanced stage-tumors, as was performed in the colorectal group.

Recently, the first evidence that abnormal MGMT expression could result in activation of an oncogene contributing to carcinogenesis was elucidated in human cancer.[12] Esteller et al.[12] demonstrated that inactivation of MGMT by promoter hypermethylation was associated with a G to A transition mutation in the K-*ras* oncogene in colorectal tumorigenesis. Thus, accumulation of this type of mutation in oncogenes or tumor suppressor genes would follow as the results of MGMT negative expression. To the best of our knowledge, few reports have described polymorphism or mutation of the human *MGMT* gene.[34–38] However, several recent reports describing negative MGMT expression have indicated that promoter hypermethylation is responsible for silencing *MGMT*. Aberrant hypermethylation is associated with a loss of MGMT protein, according to Esteller et al.[23] Epigenetic inactivation of *MGMT* may play an important role in primary human neoplasia.[39,40] Aberrant promoter hypermethylation is correlated with the loss of many cancer-related genes, such as *Rb*,[41] *p16*,[42,43] *TGFbeta-3*,[44] *E-cadherin*,[45,46] *CD44*,[47] *p73*,[48] and *hMLH1*.[49] Because the same epigenetic mechanism of negative expression could occur in the *MGMT* gene and in several other genes that play important roles in malignant potential or tumor progression, the close relationship between negative MGMT expression and the poorer prognosis in hepatocellular, gastric, and breast cancer patients could be explained.

Expression of MGMT was a predictive prognostic marker in patients with hepatocellular, gastric, and breast cancers. These findings may help to establish therapeutic strategies for patients with these types of solid cancer.

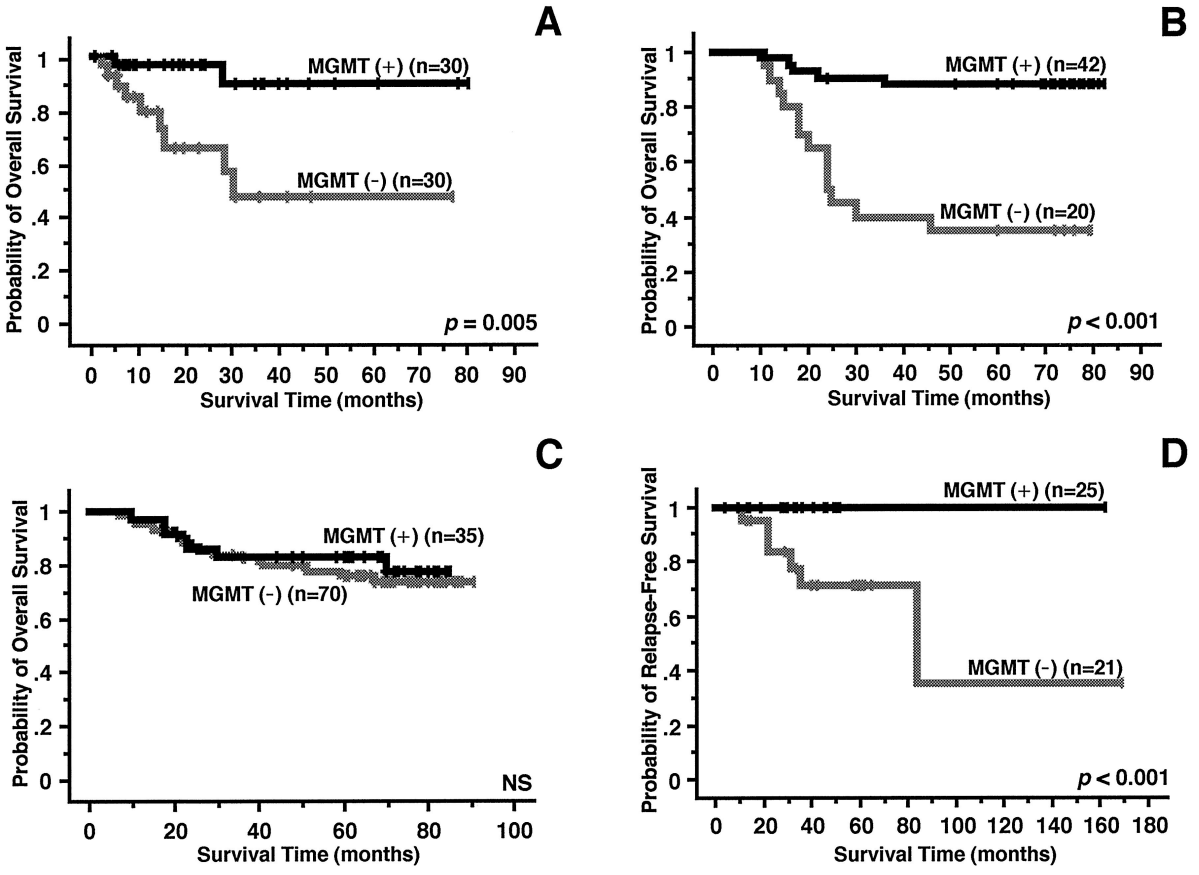

FIG. 3. Overall 5-year survival of patients with O^6-methylguanine-DNA methyltransferase (MGMT)-positive [MGMT(+)] and -negative [MGMT(−)] in hepatocellular **(A)**, gastric **(B)**, and colorectal **(C)** cancers. 5-year survival probability of MGMT(+) and MGMT(−) was 89.1% and 46.9%, respectively (log-rank test) **(A)**. 5-year survival probability of MGMT(+) and MGMT(−) was 88.0% and 35.0%, respectively (log-rank test) **(B)**. 5-year survival probability of MGMT(+) and MGMT(−) was 82.9% and 76.1%, respectively (log-rank test) **(C)**. Comparison of relapse-free survival of patients with [MGMT(+)] and [MGMT(−)] breast cancers. 10-year relapse-free survival probability was 100.0% and 35.7%, respectively (log-rank test) **(D)**.

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TABLE 5. *Multivariate analysis of Cox proportional hazards models of selected clinicopathologic variables in 62 patients with gastric cancer evaluated for prognosis*

Variable	Risk ratio for mortality (95% CI)	*P* value
Serosal invasion	0.823 (0.244–2.769)	.7526
Lymph node metastasis	9.240 (1.427–59.838)	.0196
Histological type	4.010 (0.697–23.068)	.1197
pTNM stage	14.932 (2.443–91.270)	.0034
MGMT status	0.562 (0.184–1.715)	.3116

CI, confidence interval; pTNM, pathological tumor, node, metastasis; MGMT, O^6-methylguanine-DNA methyltransferase.

REFERENCES

1. Gerson SL, Trey JE, Miller K, Berger NA. Comparison of O^6-alkylguanine-DNA alkyltransferase activity based on cellular DNA content in human, rat and mouse tissue. *Carcinogenesis* 1986;7: 745–9.
2. Wani G, Wani AA, D'Ambrosio SM. Cell type-specific expression of the *O^6-alkylguanine-DNA alkyltransferase* gene in normal human liver tissue as revealed by *in situ* hybridization. *Carcinogenesis* 1993;14:737–41.
3. Ito T, Nakamura T, Maki H, Sekiguchi M. Roles of transcription and repair in alkylation mutagenesis. *Mutat Res* 1994;314:273–85.
4. Sakumi K, Shiraishi A, Shimizu S, Tsuzuki T, Ishikawa T, Sekiguchi M. Methylnitrosourea-induced tumorigenesis in *MGMT* gene knockout mice. *Cancer Res* 1997;57:2415–8.
5. Iwakuma T, Sakumi K, Nakatsuru Y, et al. High incidence of nitrosamine-induced tumorigenesis in mice lacking DNA repair methyltransferase. *Carcinogenesis* 1997;18:1631–5.
6. Sekiguchi M, Nakabeppu Y. Adaptive response: induced synthesis of DNA repair enzymes by alkylating agents. *Trends Genet* 1987; 3:51–5.
7. Coulondre C, Miller JH. Genetic studies of the lac repressor IV.

Mutagenic specificity in the *lacI* gene of *Escherichia coli*. *J Mol Biol* 1977;117:577–606.
8. Sekiguchi M, Nakabeppu Y, Sakumi K, Tsuzuki T. DNA-repair methyltransferase as a molecular device for preventing mutation and cancer. *J Cancer Res Clin Oncol* 1996;122:199–206.
9. Ishibashi T, Nakabeppu Y, Sekiguchi M. Artificial control of nuclear translocation of DNA repair methyltransferase. *J Biol Chem* 1994;269:7645–50.
10. Mitra G, Pauly GT, Kumar R, et al. Molecular analysis of O^6-substituted guanine-induced mutagenesis of *ras* oncogenes. *Proc Natl Acad Sci U S A* 1989;86:8650–4.
11. Challen C, Lunec J, Warren W, Collier J, Bassendine MF. Analysis of the *p53* tumor-suppressor gene in hepatocellular carcinomas from Britain. *Hepatology* 1992;16:1362–6.
12. Esteller M, Toyota M, Sanchez-CM, et al. Inactivation of the DNA repair gene *O^6-methylguanine-DNA methyltransferase* by promoter hypermethylation is associated with G to A mutations in K-*ras* in colorectal tumorigenesis. *Cancer Res* 2000;60:2368–71.
13. Hall J, Bresil H, Serres M, Martel PG, Wild CP, Montesano R. Modulation of O^6-methylguanine-DNA methyltransferase in rat and hamster liver after treatment with dimethylnitrosamine. *Cancer Res* 1990;50:5426–30.
14. Nakatsuru Y, Matsukuma S, Nemoto N, Sugano H, Sekiguchi M, Ishikawa T. O^6-Methylguanine-DNA methyltransferase protects against nitrosamine-induced hepatocarcinogenesis. *Proc Natl Acad Sci U S A* 1993;90:6468–72.
15. Nakatsuru Y, Matsukuma S, Sekiguchi M, Ishikawa T. Characterization of O^6-methylguanine-DNA methyltransferase in transgenic mice introduced with the *E.coli ada* gene. *Mutat Res* 1991;254:225–30.
16. Tsuzuki T, Sakumi K, Shiraishi A, et al. Targeted disruption of the DNA repair *methyltransferase* gene renders mice hypersensitive to alkylating agent. *Carcinogenesis* 1996;17:1215–20.
17. Zaidi NH, Liu L, Gerson SL. Quantitative immunohistochemical estimates of O^6-alkylguanine-DNA alkyltransferase expression in normal and malignant human colon. *Clin Cancer Res* 1996;2:577–84.
18. Sobin LH, Wittekind C. *UICC TNM Classification of Malignant Tumors*. 5th ed. Berlin: Springer, 1997.
19. Nakabeppu Y, Nathans D. A naturally occurring truncated form of FosB that inhibits Fos/Jun transcriptional activity. *Cell* 1991;64:751–9.
20. Studier FW, Rosenberg AH, Dunn JJ. Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol* 1990;185:60–89.
21. Kawate H, Ihara K, Kohda K, Nakabeppu Y, Sekiguchi M. Mouse methyltransferase for repair of O^6-methylguanine and O^4-methylthymine. *Carcinogenesis* 1995;16:1595–602.
22. Belanich M, Randall T, Pastor MA, et al. Intracellular localization and intracellular heterogeneity of the human DNA repair protein O^6-methylguanine-DNA methyltransferase. *Cancer Chemother Pharmacol* 1996;37:547–55.
23. Estellar M, Hamilton SR, Burger PC, Baylin SB, Herman JG. Inactivation of the DNA repair gene *O^6-methylguanine-DNA methyltransferase* by promoter hypermethylation is a common event in primary human neoplasia. *Cancer Res* 1999;59:793–7.
24. Ishibashi T, Nakabeppu Y, Kawate H, Sakumi K, Hayakawa H, Sekiguchi M. Intracellular localization and function of DNA repair methyltransferase in human cells. *Mutat Res* 1994;315:199–212.
25. Fritz G, Kaina B. Genomic differences between O^6-methylguanine-DNA methyltransferase proficient (Mex^+) and deficient (Mex^-) cell lines: possible role of genetic and epigenetic changes in conversion of Mex^+ into Mex^-. *Biochem Biophys Res Commun* 1992;183:1184–90.
26. Kokkinakis DM, Ahmed MM, Delgado R, Fruitwala MM, Mohiuddin M, Albores SJ. Role of O^6-methylguanine-DNA methyltransferase in the resistance of pancreatic tumors to DNA alkylating agents. *Cancer Res* 1997;57:5360–8.
27. Major GN, Collier JD. Repair of DNA lesion O^6-methylguanine in hepatocellular carcinogenesis. *J Hepatobiliary Pancreat Surg* 1998;5:355–66.
28. Wani G, D'Ambrosio SM. Expression of the *O^6-alkylguanine-DNA alkyltransferase* gene is elevated in human breast tumor cells. *Anticancer Res* 1997;17:4311–6.
29. Pellegata NS, Sessa F, Renault B, et al. K-*ras* and *p53* gene mutations in pancreatic cancer: ductal and nonductal tumors progress through different genetic lesions. *Cancer Res* 1994;54:1556–60.
30. Luttges J, Schlehe B, Menke MA, Vogel I, Henne-Bruns D, Kloppel G. The K-*ras* mutation pattern in pancreatic ductal adenocarcinoma usually is identical to that in associated normal, hyperplastic, metaplastic ductal epithelium. *Cancer* 1999;85:1703–10.
31. Tortola S, Marcuello E, Gonzalez I, et al. *p53* and K-*ras* gene mutations correlate with tumor aggressiveness but are not of routine prognostic value in colorectal cancer. *J Clin Oncol* 1999;17:1375–81.
32. Esteller M, Gonzalez S, Risques RA, et al. K-*ras* and *p16* aberrations confer poor prognosis in human colorectal cancer. *J Clin Oncol* 2001;19:299–304.
33. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990;61:759–67.
34. Wang L, Zhu D, Zhang C, et al. Mutations of *O^6-methylguanine-DNA methyltransferase* gene in esophageal cancer tissues from Northern China. *Int J Cancer* 1997;71:719–23.
35. Otsuka M, Abe M, Nakabeppu Y, Sekiguchi M, Suzuki T. Polymorphism in the human *O^6-methylguanine-DNA methyltransferase* gene detected by PCR-SSCP analysis. *Pharmacogenetics* 1996;6:361–3.
36. Inoue R, Abe M, Nakabeppu Y, Sekiguchi M, Mori T, Suzuki T. Characterization of human polymorphic DNA repair methyltransferase. *Pharmacogenetics* 2000;10:59–66.
37. Imai Y, Oda H, Nakatsuru Y, Ishikawa T. A polymorphism at codon 160 of human *O^6-methylguanine-DNA methyltransferase* gene in young patients with adult cancers and functional assay. *Carcinogenesis* 1995;16:2441–5.
38. Rusin M, Samojedny A, Harris CC, Chorazy M. O^6-methylguanine-DNA methyltransferase (MGMT) and N-methylpurine-DNA glycosylase (MPG) in lung cancer patients from Poland. *Hum Mutat* 1999;14:269–70.
39. Danam RP, Qian XC, Howell SR, Brent TP. Methylation of selected CpGs in the human *O^6-methylguanine-DNA methyltransferase* promoter region as a marker of gene silencing. *Mol Carcinogenog* 1999;24:85–9.
40. Herfarth KK-F, Brent TP, Danam RP, et al. A specific CpG methylation pattern of the *MGMT* promoter region associated with reduced MGMT expression in primary colorectal cancers. *Mol Carcinogenog* 1999;24:90–8.
41. Stirzaker C, Millar DS, Paul CL, et al. Extensive DNA methylation spanning the *Rb* promoter in retinoblastoma tumors. *Cancer Res* 1997;57:2229–37.
42. Hermann JG, Merlo A, Mao L, et al. Inactivation of the *CDKN2/p16/MTS1* gene is frequently associated with aberrant DNA methylation in all common human cancers. *Cancer Res* 1995;55:4525–30.
43. Gonzalez ZM, Bender CM, Yang AS, et al. Methylation of the 5' CpG island of the *p16/CDKN2* tumor suppressor gene in normal and transformed human tissues correlates with gene silencing. *Cancer Res* 1995;55:4531–5.
44. Archey WB, Sweet MP, Alig GC, Arrick BA. Methylation of CpG as a determinant of transcriptional activation at alternative promoters for transforming growth factor-β3. *Cancer Res* 1999;59:2292–6.
45. Graff JR, Hermann JG, Lapidus RG, et al. *E-cadherin* expression is silenced by DNA hypermethylation in human breast and prostate carcinomas. *Cancer Res* 1995;55:5195–9.
46. Hiraguri S, Godfrey T, Nakamura H, et al. Mechanism of inacti-

vation of *E-cadherin* in breast cancer cell lines. *Cancer Res* 1998; 58:1972–7.
47. Lou W, Krill D, Dhir R, et al. Methylation of the *CD44* metastasis suppressor gene in human prostate cancer. *Cancer Res* 1999;59: 2329–31.
48. Corn PG, Kuerbitz SJ, Noesel MM, et al. Transcriptional silencing of the *p73* gene in acute lymphoblastic leukemia and Burkitt's lymphoma is associated with 5' CpG island methylation. *Cancer Res* 1999;59:3352–6.
49. Cunningham JM, Christensen ER, Tester DJ, et al. Hypermethylation of the *hMLH1* promoter in colon cancer with microsatellite instability. *Cancer Res* 1998;58:3455–60.