Clinical Analysis and prognostic Significance of the Methyl guanine Methyltransferase in Gastric Colorectal and Breast Cancers

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Abstract:

Methyl guanine – methyltransferase (MGMT) is an enzyme that repairs methyl guanine a promutagenic base damaged by endogenous and environmental alkyl ting agents. The expression of MGMT was immunohistochemically evaluated in 62, 53, and 46 paraffin-embedded samples from patients with curatively respected hepato-cellular gastric colorectal and breast cancers respectively .

The results showed that the expression of MGMT was positive predictive factor for overall survival in 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 serial 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 disease criteria's of colorectal cancers with respect to MGMT expression DID NOT SIGNIFICANTLY DIFFER .It is concluded that the expression of MGMT is a predictive prognostic marker in patients with gastric and breast cancers .these findings may help to establish therapeutic strategies for patients with these types of solid cancer.

Introduction:

Environmental alkyl ting agents such as compounds are principally metabolized and activated in hepatocytes [1, 2]. Because endogenous alkyl ting compounds are released into bile and the digestive tract epithelial cells in the billiard and gastrointestinal tract are always exposed to activated alkyl ting agents [2]. Alkyl ting agents cause gene mutations or cell death in vitro [3]. and carcinogenesis or apoptosis in vivo [4, 5]. These biological effects are induced by promutagenic base [6] methylgunine which is produced by the alkyl ting agents [6]. Methyl guanine preferentially impairs with thymine instead of cytosine during DNA replication leading to a G: C/A: T transition mutation [7]. Humans possess methyl guanine- DNA methyltransferase (MGMT) which repairs methyl guanine to prevent such misplaying [8]. Abnormal MGMT expression causes methyl guanine to accumulate in cellular DNA [9] and this could result in activation of ontogenesis or inactivation of tumor suppressor genes contributing to carcinogenesis or tumor progression [10, 12]. Recent findings from animal models and in vitro studies demonstrate that a deficiency in methyl guanine repair seems to be one major determinate of susceptibility to carcinogenesis by alkyl ting 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, and 16]. If activation of ontogenesis 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 the tumor grade or the prognosis are scarce. Saudi et al [17]. reported that MGMT immunohisto-chemical staining correlates with protein quantity and activity .thus immunohisto-chemical can determine both the expression and distribution of MGMT protein .this study investigates the relationship between negative expression of MGMT determined by immunohistochemical and clinic pathologic

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features including prognosis to clarify whether or not abnormal MGMT expression participates in the carcinogenesis and tumor progression of gastric colorectal and breast cancers.

Materials and methods:

Patients with primary gastric cancer colorectal cancer and breast cancer admitted to the department of surgery ,hill teaching hospital medical collage were considered for inclusion in this study included consecutive of 62 ,53 and 46 patients with priory gastric colorectal and breast from 1991 to 2002 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 record and by contacting patients and physicians the mean follow-up period of patients with gastric colorectal and cancer was 46 months (range,5 to 80) representative –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].

We used commercialized anti-human MGMT [19, 15]. antibody the MGMT custom-made antibody actions(5 um) were deparaffinized in xylem and dehydrated antigen was retrieved by micro waving the samples three times for 5 minutes in 10 mom of sodium citrate buffer (ph6.5) . Endogenous peroxides 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 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 goat anti-rabbit immunoglobulin which were conjugated with peroxides –labeled dextral polymer at room temperature for 30 minutes .the slides were washed in phosphate –buffered saline twice and developed by using a DAB substrate 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 .the status of MGMT expression as positive or negative was assessed .the sample was considered positive when immunoreactivity was detected in>10% of the cells in nuclei ,cytoplasm ,or both .[22-25.]

Statistical analysis:

The clinic pathologic characteristics were compared with MGMT positive and negative groups and the significance of associations was determined with the man Whitney u-test or student's t-test for continuous data and the x^2 test categorical data. The survival data were used to Generate Kaplan-Meier curves that were compared on the basis of MGMT status by using the log-rank test .statistical significance was judged as $p<.05$.

Results:

MGMT proteins were detected in normal epithelia interstitial fibroblasts vascular smooth muscle and the smooth muscle layer tumors positive for MGMT homogeneously expressed the protein in the nuclei gastric cancer (fig.1) colorectal cancer (fig.2) and breast cancer cells (fig.3) several cancers in which signals for the protein were detected in the cytoplasm were classified as MGMT positive cancer tumors without signals for the nuclei and cytoplasm were defined as MGMT negative cancer interstitial fibroblast was shown as an internal positive control MGMT positivist was identified in 67.7%(42 patients) of 62 gastric cancers 33.3%(35 patients) of 53 colorectal cancers and 54.3% (25 patients)of 46 breast cancers.

Correlation between mgmt expression ststus and clinicopathologic features:

In gastric cancer serial invasion limp [h node metastasis histological 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 1.

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 clinic pathologic features analyzed in the study did not significantly correlate the overall 5-years survival rates for patients with MGMT positive and negative tumors were 88.0% and 35.0% respectively (p<.001).

In colorectal cancer the MGMT expression status and age sex tumor size tumor location lymphatic invasion venous serial invasion lymph node metastasis grade of differentiation and pathologic tumor node metastasis stage of the tumors did not significantly correlate (table2) 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) All of breast cancers were pathologically diagnosed as invasive ducal carcinoma because only two patients with breast cancer died wee analyzed not overall survival but relapse-free survival rates local recurrence of MGMT –negative tumors was frequent with a significant a significant difference (p=004,table3) the MGMT expression status and the other clinic pathologic characteristics not significantly correlate the 10-year relapse free survival rates for patients with MGMT positive and negative breast cancers were 100.0% and 35.7% respectively (p<001).

To determine the variables affecting the survival of gastric cancer patients, five variables correlated in university analysis (serial invasion, lymph node metastasis histologic type pathological 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 Postoper-tive survival

Table 1: correlation of MGMT expression with clinic pathologic features In gastric cancer

Patient &tumor characteristics	No. patients	MGMT		P value [a]
		Positive (%)	Negative (%)	
Total tumors	62	42(67.7)	20(32.3)	
Agley(mean # SD)	65 # 6	66 # 3	65 # 5	NS
Sex(male: female)	46:16	43:01	14:6	NS
Tumor diametric(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)	
Serial 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				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				018
Intestinal	38	30(78.9)	8(21.1)	
Diffuse	24	12 (50.0)	12(50.0)	
PTNM stage				< .001
1A	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)	
Snoot statistically significant, MGMT,methylguanine –methyltransferase				
P value calculated by X 2 test and Mann Whitney U-tests for comparison of positive and negative groups.				

Table2.correlation of mgmt expression with clinicopathologic features In colorectal cancer

Patient and tumor characteristics	No. patients	MGMT	
		Positive (%)	Negative (%)
Total tumors	55	35(33.3)	70(66.7)
Agley (mean# SD)	68#7	67#6	69#8
Sex (male :female)	64:41	22:13	42:28
Tumor diameter ,cm (mean's)	6.6#3.0	6.5#2.6	6.4#2.3
Tumor location			
Colon	33	10(30.8)	23(69.2)
RECTUM	20	7(37.5)	13(62.5)
Lymphatic invasion			
Absent	23	9(37.8)	14(62.2)
Present	30	9(30.0)	21(70.0)
Venous invasion			
Absent	9	3(38.9)	6(61.1)
Present	44	14(32.2)	30(67.8)
Absent	41	31(30.9)	28(69.1)
Present	12	5(41.7)	7(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)
P TNM stage a			
I	25	8(32.0)	17(68.0)
II	37	11(26.7)	26(70.3)
III	35	13(37.1)	22(62.9)
IV	8	3(37.5)	5(62.5)

TABLE 3: correlation of mgmt expression with clinic pathologic 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's)	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				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)	
P TNM stage				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)	
Snoot statistically significant , MGMT ,nethylguanine-methyltransferase,p TNM , pathological tumor ,node ,metastasis				
a P value calculated bx-2 test and Mann Whitney U-tests for comparison of positive and-negative groups				

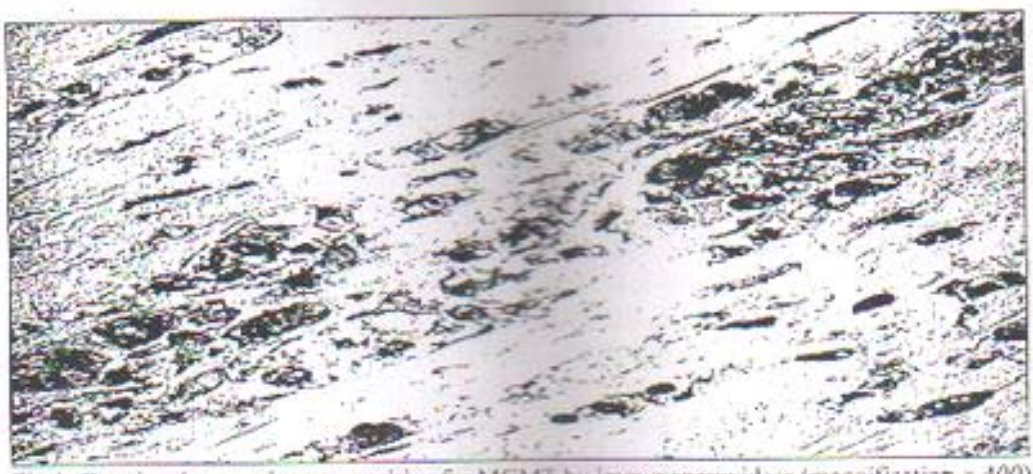

Fig 1. Gastric adenocarcinoma , positive for MGMT by immunoperoxidase (magnification × 400).

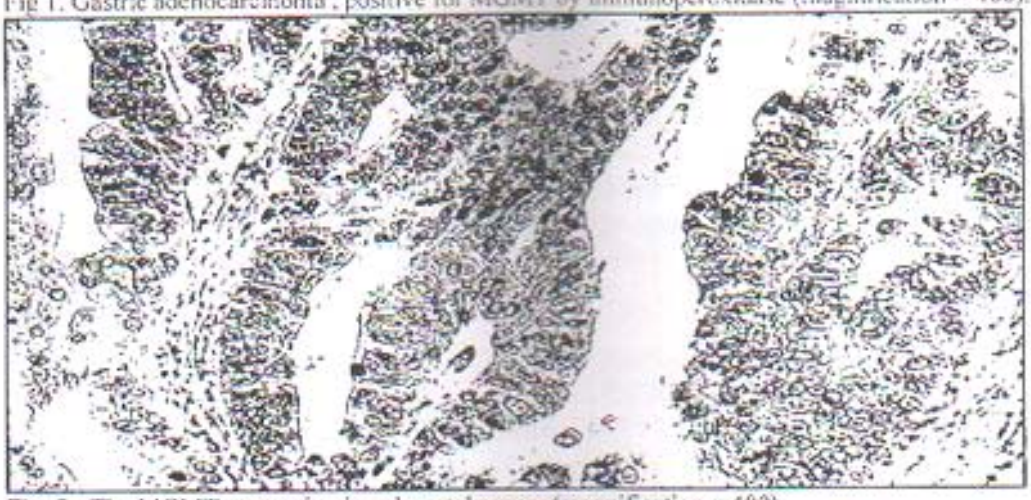

Fig. 2. The MGMT expression in colorectal cancer (magnification × 400).

Fig 3, breast cancers positive for MGMT ,nonogeneously expressed in the nuclei (magnification × 200).

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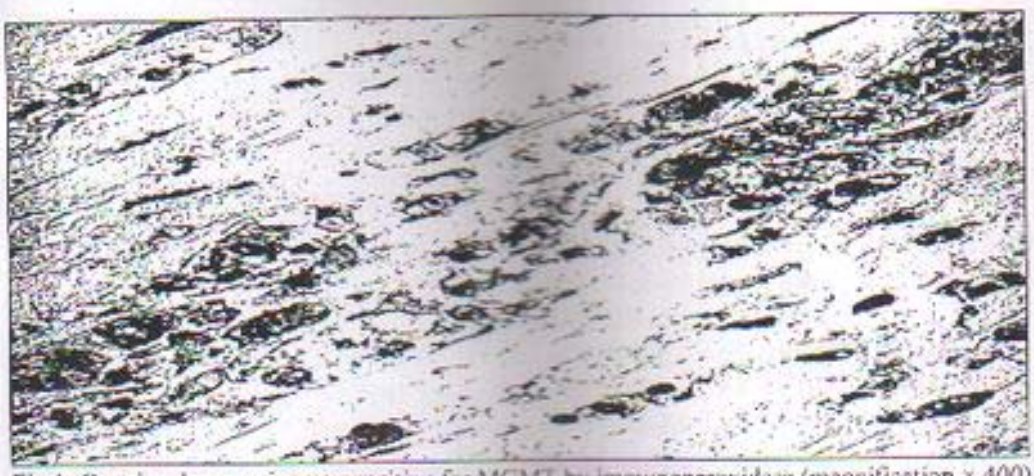

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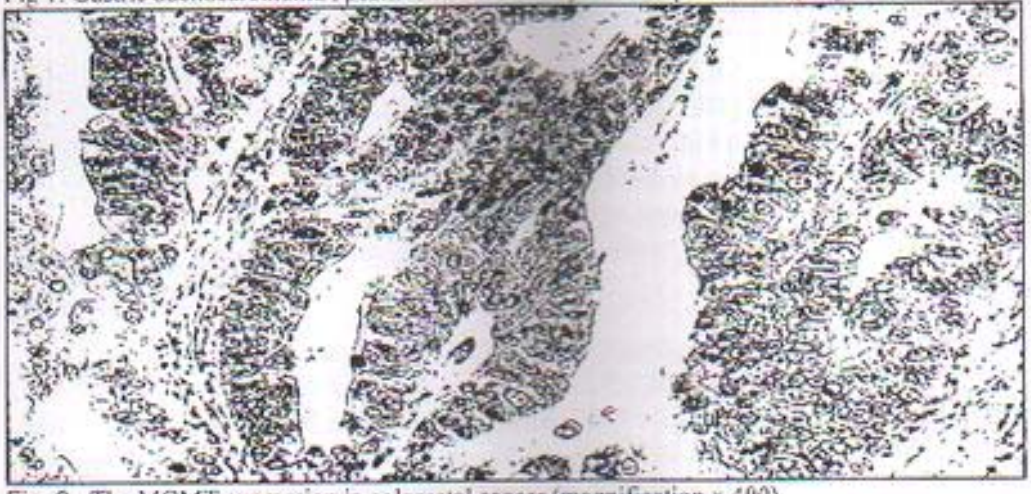

Fig. 2. The MGMT expression in colorectal cancer (magnification × 400).

Fig 3, breast cancers positive for MGMT ,homogeneously expressed in the nuclei (magnification × 200).

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11

Discussion:

The expression of MGMT protein has been studied kokkinakis 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 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 ducal Aden carcinoma 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.

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 stag was the strongest prognostic marker. Thus abnormalities related to cancerous invasion and metastasis such as adhesion molecules, shoulde are involved in MGMT dysfunction.

In a study of breast cancer however wane and d'ambrosio [28.] reported that tumor grade and metastasis potential of breast cancer were not correlated with negative expression of the messenger RAN 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 variations 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] has k-raps

Mutations k-raps 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 ontogeny contributing to carcinogenesis was elucidated in human cancer [12]. demonstrated that inactivation of MGMT by promoter hypermethylation was associated with G to A transition mutation in the K-raps ontogeny in colorectal tumor genesis .thus accumulation of this type of mutation in ontogenesis or tumor suppressor genes would follow as the results of MGMT negative expression previous reports have described polymorphism or the human MGMT gene [34,38].however several recent reports describing negative MGMT expression have indicated that promoter hypermethylations is responsible for silencing MGMT

Conclusion:

An aberrant hypermethylation is associated with a loss of MGMT protein according to stellar et al [23.] Epigenetic inactivation of MGMT may play an important in primary human neoclassic [39, 41].

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 gastric and breast cancer patients could be explained. Therefore expression of NGNT was a predictive prognostic marker in patients with gastric and breast cancers these findings may help establish therapeutic strategies for patients with these types of solid cancer.

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