GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Association of MLL3 expression with prognosis in gastric cancer

B. Li, H.Y. Liu, S.H. Guo, P. Sun, F.M. Gong and B.Q. Jia

Department of Surgical Oncology,
General Hospital of the People's Liberation Army, Beijing, China

Corresponding author: B.Q. Jia
E-mail: jiabaoqing1971@126.com

Genet. Mol. Res. 13 (3): 7513-7518 (2014)
Received July 25, 2013
Accepted June 26, 2014
Published September 12, 2014
DOI http://dx.doi.org/10.4238/2014.September.12.18

ABSTRACT. Low expression of myeloid/lymphoid or mixed-lineage leukemia 3 (MLL3) is reportedly associated with gastric cancer and tumor progression. The purpose of this study was to examine the expression of MLL3 in tissue samples of patients with gastric cancer and to analyze the relationship between MLL3 protein expression and clinical records. Using immunohistochemical staining and Kaplan-Meier analysis for MLL3 in gastric cancer patients, we found that low expression of MLL3 had a significant relationship with a low survival rate compared to positive MLL3 expression in the patients analyzed ($P < 0.05$). Our data suggest that MLL3 expression plays a vital role in gastric cancer development, and that this protein is an important marker for the prognosis of gastric cancer.

Key words: MLL3; Survival; Gastric cancer

INTRODUCTION

Although the incidence and mortality of gastric cancer has gradually decreased in East Asia, the disease remains the most common malignant tumor in China (Meng et al., 2013). In addition, gastric cancer is difficult to diagnose at early stages. Treatments such as surgery, irradiation therapy, and chemotherapy for gastric cancer patients have improved in recent years, but overall survival has remained nearly unchanged and the prognosis is disappointing. Prognosis is also significantly different for various gastric cancer patients, even those in the same tumor node metastasis (TNM) stage. Recent studies have focused on identifying molecular markers and developing related active treatment methods for gastric cancer. Efforts have been made to identify biomarkers for predicting survival or recurrence in gastric cancer (Wang et al., 2013; Sezer et al., 2013; Yu et al., 2013).

The myeloid/lymphoid or mixed-lineage leukemia 3 (MLL3) gene is a member of the trithorax (TRX)/MLL gene family and maps to chromosome 7q36.1. It encodes a predicted protein of 4911 amino acids containing 2 plant homeodomains (PHD), an ATPase alpha/beta signature, a high-mobility group, a suppressor of variegation, enhancer of zeste, trithorax (SET), and two phenylalanine and tyrosine (FY)-rich domains. PHD and SET domain proteins are chromatin regulators, and several are altered in cancer (Saha et al., 1995). Inactivation of MLL3 in mice results in epithelial tumor formation, suggesting that *MLL3* functions as a tumor-suppressor gene (Lee et al. 2009). In addition, *MLL3* has been reported to be frequently deleted in myeloid leukemia (Ruault et al., 2002). Moreover, other studies have reported somatic mutations in the *MLL3* gene in glioblastoma and pancreatic ductal adenocarcinoma (Balakrishnan et al., 2007).

In this study, we examined MLL3 expression in 90 cases of gastric cancer patients through streptavidin-biotin peroxidase (SP) immunohistochemical staining and conducted survival analysis for these 90 patients over a follow-up period of 9-120 months after surgery.

MATERIAL AND METHODS

Patients and samples

Between January 2002 and December 2003, 90 patients with advanced gastric cancer undergoing curative resection were recruited from the Department of Pathology in the General Hospital of the People's Liberation Army. The follow-up information was complete for all patients. Thirty-two patients were female and 58 were male; their ages ranged from 30 to 77 years. All clinical data were sorted by the tissue type, lymph node metastasis, and TNM stage according to the New Standard of Prognosis in Common Malignant Tumors (AntiCancer AoC, 1999) criteria (Table 1). Of the tumors that arose in the stomach, 50 cases involved the gastric antrum, 17 cases involved the lesser curvature, 9 cases involved the cardia of the stomach, 8 cases involved the gastric fundus, and 6 cases involved the gastric body.

Immunohistochemistry

All tumor tissue specimens were routinely fixed in 10% formalin and embedded in paraffin. Specimens were selected for immunohistochemistry of MLL3. The rabbit polyclonal anti-human MLL3 antibody (SantaCruz Biotechnology; Santa Cruz, CA, USA) was used. The

SP complex method was employed for immunohistochemical steps and was conducted using a commercially available SP kit (Beijing Zhongshan Jinqiao Biotechnology Limited Company; Beijing, China). Phosphate-buffered saline (PBS) rather than primary antibody was used as a negative antibody control in the SP immunohistochemical staining analysis. After deparaffinization, slides were rehydrated in graded alcohols and placed in PBS, pH 7.4, solution. Antigen retrieval was conducted by autoclaving the slides in citrate buffer, pH 6.0. Endogenous peroxidase activity was quenched by dipping in 3% H_2O_2 for 10 min and washing with PBS, pH 7.4, 3 times. Nonspecific binding was blocked by treating slides with 5% goat serum. Slides were treated with rabbit polyclonal anti-human MLL3 antibody (1:100 dilution), mouse monoclonal anti-human surviving antibody (ready to use), biotinylated goat anti-rabbit/anti-mouse/anti-rice/anti-guinea IgG, and streptavidin-peroxidase, with washing 3 times in PBS, pH 7.4, between each step. The sites of peroxidase binding were visualized using diaminobenzidine. Sections were counterstained lightly with hematoxylin.

Assessment of MLL3 immunostaining in carcinomas

Evaluation criteria of MLL3 in gastric cancer are described in the methods for semi-quantitative integration (Tang et al., 2008). Immunohistochemistry results were analyzed to generate a histoscore that combines the intensity of the immunoreaction with the number of positive cells. Five visual fields for each sample were chosen randomly, and the positive cells in each visual field were counted. Briefly, the intensity of staining was scored as follows: 0 for non-staining, 1 for yellow staining, 2 for brown-yellow staining, and 3 for brown staining. The number of positive cells was scored as 0 when ≤5% of tumor cells had positive staining; 1, 6-25% of tumor cells had positive staining; 2, 26-50 % of tumor cells had positive staining; 3, 51-75 % of tumor cells had positive staining; and 4, ≥75 % tumor cells had positive staining. The final histoscores obtained with the 2 scales of staining score were multiplied and treated as negative (-) when the score was 0; weakly positive (+) when the score was 1-4; moderately positive (++) when the score was 5-8; and strongly positive (+++) when the score was 9-12. For statistical analysis, a tumor was considered to be positive if it demonstrated any degree of MLL3 staining.

Statistical methods

The SPSS16.0 software was used for statistical analysis (SPSS, Inc.; Chicago, IL, USA). A P value of <0.05 was considered to be statistically significant. The association between clinico-pathological characteristics and MLL3 expression and survival rate was analyzed by pairwise χ^2 tests. To investigate the potential associations between biomarkers and survival, multivariate binary logistic regression analysis was conducted. Lifetables and Kaplan-Meier survival curves were used to perform the survival analysis.

RESULTS

Expression of MLL3 in gastric cancer tissues

After analyzing 90 gastric cancer tissues by immunohistochemical staining, positive expression of MLL3 was observed in the cytoplasm of cancer cells (Figure 1). Of the 90 speci-

mens, 48 tissues showed positive expression of MLL3, accounting for 54.4% of the total analyzed amounts (Table 1). Among the 48 tissues showing MLL3-positive expression, 30 cases showed positive MLL3 expression that was weakly positive (+), 12 cases were moderately positive (++), and 6 cases were strongly positive (+++) (data not shown).

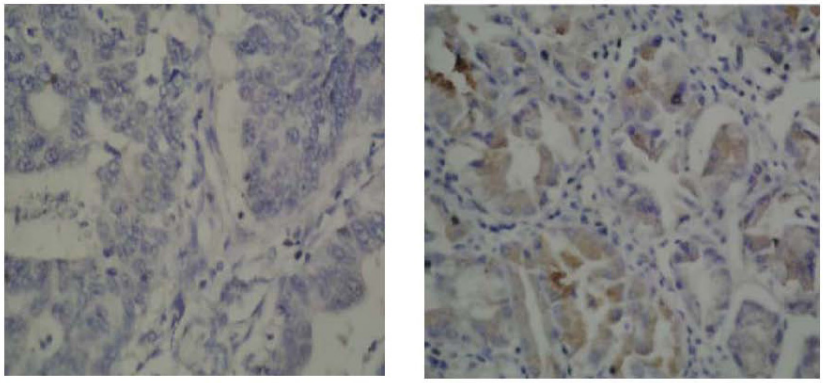

Figure 1. Myeloid/lymphoid or mixed-lineage leukemia (MLL3) expression detected by SP immunohistochemical staining in gastric cancer patient tissue samples. **A.** MLL3-negative expression; **B.** MLL3-positive expression.

Table 1. MLL3 expression and clinical pathology factors.

Item	Cases (N)	MLL3	
		Positive (N)	P
Gender			
Men	58	31	0.85
Women	32	17	
Age (years)			
<60	25	16	0.42
>60	65	32	
Infiltration			
T1	10	3	0.086
T2	11	5	
T3	44	22	
T4	25	18	
Lymph node metastasis			
No	21	14	0.003
Yes	69	34	
Distant metastasis			
No	76	46	0.001
Yes	14	2	
TNM stage			
I	10	8	0.012
II	28	20	
III	37	13	
IV	15	7	

Survival analysis after surgery for gastric cancer patients

Since positive expression of MLL3 was observed in gastric cancer samples, we exam-

ined the relationship between MLL3 protein expression and survival rate for all the 90 gastric cancer patients. Survival rates of 3, 5, and 8 years for the 90 patients were 52.2% (47 patients), 46.8% (34 patients), and 10.3% (5 patients), respectively. Notably, survival rates showed a sharp decline among all patients during the first 3 years and between 5 and 8 years, suggesting that most patients developed malignant gastric cancer and died soon after diagnosis within the first 3 years (nearly 50% of patients) or after diagnosis from 5 to 8 years (>35% of patients).

The relationship between MLL3 expression and survival rates for different periods of time after surgery revealed that the overall survival of all patients with MLL3-positive tumors was significantly greater than that for patients with MLL3-negative tumors ($P < 0.001$). Moreover, patients with MLL3-negative expression showed a dramatic decrease in survival rate at approximately 2 years after surgery, while patients with MLL3-positive expression showed a fast decrease in survival approximately 5 years after surgery.

DISCUSSION

The etiology and pathogenesis of gastric cancer is very complex and is not fully understood. Gastric cancer is now considered to result from multiple factors, such as lifestyle, diet, genetics, *Helicobacter pylori* infection, chronic gastritis, gastric dysplasia, intestinal metaplasia, and surgical injury. *H. pylori* infection and genetic factors are the most important factors for gastric cancer. Because of the lack of simple and effective diagnostic methods for early diagnosis, the optimal treatment window is often missed. Therefore, in-depth study of gastric cancer development and the molecular mechanisms of metastasis to identify molecular markers for early diagnosis and drug targets have received significant attention.

MLL3 is a histone methyltransferase (COMT) that specifically methylates the 4th lysine in histone 3. Recent studies have shown that MLL3 mutation and low expression are common phenomena in gastric cancer. In addition, the gastric cancer cell genome shows that instability, particularly microsatellite instability (MSI), is increasing. In rectal cancer, MLL3 was found to be expressed at abnormal levels. An MLL3 mutation leading to a loss of function or reduced expression in gastric cancer may be a key factor resulting in gastric epithelial cell genomic instability and thus promote gastric carcinogenesis.

The results of this study showed that expression of MLL3 in gastric cancer may be associated with patient survival after curative resection and that MLL3 expression was an independent predictor of disease recurrence. In addition, low MLL3 expression was correlated with a lower survival rate, and survival rates were correlated with most clinical pathology factors. Therefore, MLL3 expression plays a vital role in gastric cancer development and may be an important marker for the diagnosis and prognosis of gastric cancer.

REFERENCES

Anti-Cancer AoC (1999). Diagnosis Rules for New Common Malignant Tumors. Peking Union Medical College Press, Beijing.

Balakrishnan A, Bleeker FE, Lamba S, Rodolfo M, et al. (2007). Novel somatic and germline mutations in cancer candidate genes in glioblastoma, melanoma, and pancreatic carcinoma. *Cancer Res.* 67: 3545-3550.

Lee J, Kim DH, Lee S, Yang QH, et al. (2009). A tumor suppressive coactivator complex of p53 containing ASC-2 and histone H3-lysine-4 methyltransferase MLL3 or its paralogue MLL4. *Proc. Natl. Acad. Sci U. S. A.* 106: 8513-8518.

Meng JR, Tang HZ, Zhou KZ, Shen WH, et al. (2013). TFF3 and survivin expressions associate with a lower survival rate in gastric cancer. *Clin. Exp. Med.* 13: 297-303.

Miyazaki K, Inokuchi M, Takagi Y, Kato K, et al. (2013). EphA4 is a prognostic factor in gastric cancer. *BMC. Clin. Pathol.* 13: 19.

Ruault M, Brun ME, Ventura M, Roizes G, et al. (2002). MLL3, a new human member of the TRX/MLL gene family, maps to 7q36, a chromosome region frequently deleted in myeloid leukaemia. *Gene* 284: 73-81.

Saha V, Chaplin T, Gregorini A, Ayton P, et al. (1995). The leukemia-associated-protein (LAP) domain, a cysteine-rich motif, is present in a wide range of proteins, including MLL, AF10, and MLLT6 proteins. *Proc. Natl. Acad. Sci U. S. A.* 92: 9737-9741.

Sezer C, Yildirim M, Yildiz M, Sezgin A, et al. (2013). Prognostic significance of biological apoptosis factors in gastric cancer. *J. BUON.* 18: 138-146.

Tang ZH, Chen HS, Zou ZK, Cai SB, et al. (2008). Trefoil factor 4 and CD147 expression in carninogenesis of gastric mucosa and their correlation with angiogenesis. *World Chin. J. Digestol.* 16: 3631-3636.

Wang G, Wang W, Zhou J and Yang X (2013). Correlation between telomerase activity and matrix metalloproteinases 2 expression in gastric cancer. *Cancer Biomark* 13: 21-28.

Yu JW, Wu SH, Lu RQ, Wu JG, et al. (2013). Expression and significances of contactin-1 in human gastric cancer. *Gastroenterol. Res. Pract.* 2013: 210205.