Int J Clin Exp Pathol 2015;8(11):14864-14874
www.ijcep.com /ISSN:1936-2625/IJCEP0016251

Original Article

DNA methyltransferase 3a rs1550117 genetic polymorphism predicts poor survival in gastric cancer patients

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Received September 15, 2015; Accepted October 23, 2015; Epub November 1, 2015; Published November 15, 2015

Abstract: DNA methyltransferase 3a (DNMT3a) have been suggested to play a crucial role in human cancer prognosis. Single nucleotide polymorphisms (SNPs) in *DNMT3a* genes may have an impact on the prognosis of cancers. This study aimed to investigate the association between SNPs of *DNMT3a* gene and prognosis of gastric cancer (GC). Two sites of *DNMT3a* SNPs, rs1550117 and rs13420827 were selected and genotyped using TaqMan assay in 447 GC patients who received gastrectomy. Effects of genotypes on clinical outcomes of GC were calculated by Kaplan-Meier survival analysis and Cox regression model. We found that the AG or AA genotype of rs1550117 was associated with significantly poorer survival and increased death risk of GC compared with GG genotype (dominant model: HR=1.35, 95% CI=1.01-1.80, *P*=0.043). Further multivariate Cox regression analysis revealed that in addition to the known factors including male, larger tumor sizes and high clinical stage, rs1550117 variant was an independently predictive factor for survival in GC patients. No significant association was found between rs13420827 genetic variants and GC prognosis. Our findings first demonstrated that *DNMT3a* rs1550117 polymorphism may be a potential biomarker in predicting overall survival of GC patients.

Keywords: DNA methyltransferase 3a, gastric cancer, polymorphisms, overall survival

Introduction

Gastric cancer (GC) is the second leading cause of cancer-related death and the fourth most common malignancies in the world [1]. Despite recent improvements in diagnosis and treatment for GC, the 5-year overall survival (OS) rate remains low at 30% [2]. In consideration of the therapeutic effect, TNM (tumor-node-metastasis) staging, as the most acceptable clinical measurement, has been widely used to evaluate the prognosis of GC. However, due to individual differences, patients with the same TNM stage may have various outcomes. Researchers have realized that TNM staging alone has its limitation in predicting prognosis of cancer [3, 4]. Therefore, it is critical to identify more prognostic biomarkers that could be helpful in the improvement of screening of high-risk individuals, early diagnosis, as well as predicting outcome for the individualized therapy [5]. In recent years, increased studies have focused on exploring genetic variants that could have an impact on gastric cancer risk and progression [6-9].

DNA methylation is one of the epigenetic modulations and plays an essential role in the development of tumors [10]. Aberrant DNA methylation is closely associated with increased risk of a series of human cancers, including gastric cancer [11]. Aberrant DNA methylation could result in both genome-wide hypomethylation and regional hypermethylation of CpG islands. Hypermethylation of CpG sequences has been identified as a possible mechanism for silencing of tumor suppressor genes in human cancers [12-14]. Three active forms (*DNMT1*, *DNMT3a* and *DNMT3b*) of DNA methyltransferase have been identified, which are responsible

for mediating DNA methylation during biological processes [15]. DNA methyltransferase 3a (DNMT3a) is the enzyme involved in *de novo* methylation which is essential for the generation of genomic methylation patterns and germ line development during embryogenesis [16, 17]. Previous studies have shown that single nucleotide polymorphism (SNPs) of *DNMT3a* were correlated with the susceptibility of gastric cancer [18, 19] and colorectal cancer [20] but not in hepatocellular carcinoma [21] and ovarian cancer [22].

Early studies demonstrated that DNMTs are overexpressed in a variety of cancers, implying that DNMTs may be involved in the carcinogenesis [23, 24]. Our previous study found that positive expression of DNMT3a is an independent poor prognostic indicator in gastric cancer [25]. These studies revealed the possible role of DNMT3a in the development and progression of tumors. Furthermore, genetic variations in *DNMT3a* have been shown to be prognostic predictors for patients with acute myeloid leukemia (AML). For example, a study by Ribeiro *et al*. [26] reported that mutations in *DNMT3a* (at position R882) independently predicted a shorter overall survival of acute myeloid leukemia (AML) in Netherlands patients. Similar results were found in Chinese groups that *DNMT3a* mutation status was associated with an unfavorable prognosis [27, 28].

However, as far as we know, no study was available on the role of *DNMT3a* polymorphism in the prognosis of gastric cancer. Considering the evidence implicating the role of *DNMT3a* in promoting tumorigenic processes, it is highly plausible that *DNMT3a* polymorphisms could harbor functional genetic variants that may help define effects on the progression and prognosis of gastric cancer. In the present study, we analyzed the common genetic polymorphisms in the *DNMT3a* gene, and attempted to elucidate the association between the selected two SNPs and clinical significance as well as overall survival in Chinese gastric cancer patients.

Materials and methods

Study population

This study was approved by the Ethics Committee of the First Hospital of Jilin University (Changchun, Jilin, China). Written informed consents were obtained from all the subjects prior to taking part in this research. A total of 447 gastric cancer patients who underwent a surgical resection were recruited at the Department of Gastric and Colorectal Surgery, First Hospital of Jilin University (Changchun, China) between 2008 and 2010. All patients were newly diagnosed, histopathologically confirmed with GC and they were treated with surgery alone. None of the cases were received chemotherapy or radiotherapy prior to surgery. We defined chemotherapy as an effective treatment for at least 3 cycles. The patients received several postoperative chemotherapy regimens according to NCCN Clinical Practice Guidelines in Oncology for Gastric Cancer, version 2008-version 2010. The tumor histological grade was evaluated according to the 2002 International Union against Cancer (UICC) TNM classification system (the 6th edition) and WHO histological criteria. Complete clinical data such as age, sex, history of tumor were obtained from the medical records. In addition, 5 ml venous blood was donated by all patients, collected in tubes containing EDTA-anticoagulant and stored at -80°C until genomic DNA extraction.

Regular follow-up and outcomes collection

Gastric cancer cases were followed-up by telephone calls three month, six month, and one year after the tumorectomy and every one year later until death. The survival time was defined as the duration from the date of surgical operation to the date of death if the patients were died or to the date of the last successful interview if the patients were lost to follow-up or alive until the end of the study. Anyone meet the following criteria would not include in the survival analysis: (i) they were lost to follow-up at the first time of telephone interview, or (ii) they were died of complications of the surgical operation in the perioperative period, (iii) patients had received chemotherapy or radiotherapy prior to surgery.

SNP selection

The principal hypothesis underlying this study is that one or more SNPs in the *DNMT3a* regions are associated with risks of gastric cancer. Thus, the aim of SNP tagging is to identify a set of SNPs that may be surrogates for many thousands of other SNPs. Among the candidate

Table 1. Clinical data of gastric cancer patients

Variables	Patients (n %)	Deaths (n %)	MST	Log-rank P	HR (95% CI)
Age					
<60	196 (46.4)	86 (44.1)	66.77	0.377	1.00
≥60	226 (53.6)	109 (55.9)	61.80		1.14 (0.86-1.51)
Gender					
Male	305 (72.3)	151 (77.4)	46.98*	0.041	1.00
Female	117 (27.7)	44 (22.6)	54.25		0.71 (0.51-0.99)
Tumor sizes					
<5 cm	231 (55.8)	76 (40.2)	58.90*	<0.001	1.00
≥5 cm	183 (44.2)	113 (59.8)	37.68		2.52 (1.89-3.38)
TMN stages					
I	66 (15.6)	5 (2.6)	72.53*	<0.001	1.00
II	174 (41.3)	51 (26.2)	61.61*		4.28 (1.71-10.73)
III	152 (36.0)	116 (59.4)	27.94		19.66 (8.02-48.23)
IV	30 (7.1)	23 (11.8)	23.84		21.61 (8.20-56.95)
Tumor differentiation					
Well	155 (37.1)	69 (35.9)	66.77	0.160	1.00
Moderate/poor	263 (62.9)	123 (64.1)	47.30*		1.24 (0.92-1.66)
Lauren classification					
Diffuse	31 (7.4)	19 (9.7)	28.20	0.140	1.00
Intestinal	368 (87.4)	168 (86.2)	49.59*		0.65 (0.40-1.05)
Mixed	22 (5.2)	8 (4.1)	59.43		0.50 (0.22-1.15)
Depth of invasion					
T1	43 (10.3)	4 (2.1)	71.97*	<0.001	1.00
T2	54 (12.9)	8 (4.1)	70.29*		1.63 (0.49-5.40)
T3	303 (72.3)	165 (85.5)	36.17		8.26 (3.06-22.29)
T4	19 (4.5)	16 (8.3)	11.40		19.82 (6.61-59.42)
Lymph node metastasis					
N0	119 (28.4)	14 (7.3)	70.23*	<0.001	1.00
N1	122 (29.1)	46 (23.8)	56.20*		3.74 (2.05-6.80)
N2	91 (21.7)	58 (30.1)	23.60		8.50 (4.73-15.26)
N3	87 (20.8)	75 (38.8)	9.83		19.65 (11.03-35.02)
Distant metastasis					
M0	392 (92.9)	172 (88.2)	50.81*	<0.001	1.00
M1	30 (7.1)	23 (11.8)	13.27		2.75 (1.78-4.26)
Chemotherapy					
XELOX[1]	29 (6.9)	9 (4.6)	54.75*	0.196	1.00
FOLFOX-4[2]	77 (18.2)	36 (18.5)	45.70*		1.74 (0.84-3.60)
Others[3]	32 (7.6)	20 (10.3)	38.41		2.35 (1.07-5.16)
None	284 (67.3)	130 (66.7)	49.10		1.73 (0.88-3.39)

MST: median survival time. HR: hazard ratio. *Mean OS was presented when median OS could not be calculated. [1]XELOX (capecitabine and oxaliplatin). [2]FOLFOX-4 (5-fluorouracil, leucovorin and oxaliplatin). [3]Other chemotherapies included: 5-fluorouracil; xeloda alone; paclitaxel plus leucovorin and tegafurum; LV5-FU2 (leucovorin plus 5-fluorouracil); FOLFIRI (irinotecan, 5-fluorouracil and leucovorin).

SNPs in *DNMT3a*, common and potentially functional polymorphisms within the *DNMT3a* genes were selected in the HapMap Project using SNPbrowser Software v4.0 (06-02-2009, www.hapmap.org). SNPs were selected based on the following criteria: (i) minor allele frequency (MAF) ≥0.05 and a pair-wise r^2 of 0.8 or greater in Chinese Han Population; (ii) locate in

Table 2. Distributions of genotypes according to clinical parameters in gastric cancer cases

Characteristics	rs1550117		*P* value	rs13420827		*P* value
	GG (n %)	AG/AA (n %)		CC (n %)	CG/GG (n %)	
N	272 (64.5)	150 (35.5)		280 (66.4)	142 (33.6)	
Age	61 (53-70)	61 (54-70)	0.276	61 (54-71)	60 (53-69)	0.090
Gender						
Male	195 (63.9)	110 (36.1)	0.718	198 (64.9)	107 (35.1)	0.315
Female	77 (65.8)	40 (34.2)		82 (70.1)	35 (29.9)	
H.pylori						
Negative	83 (64.8)	45 (35.2)	0.912	90 (70.3)	38 (29.7)	0.256
Positive	189 (64.3)	105 (35.7)		190 (64.6)	104 (35.4)	
Tumor size						
<5 cm	152 (65.8)	79 (34.2)	0.394	156 (67.5)	75 (32.5)	0.443
≥5 cm	113 (61.7)	70 (38.3)		117 (63.9)	66 (36.1)	
Differentiation						
Poor	104 (67.1)	51 (32.9)	0.369	100 (64.5)	55 (35.5)	0.561
Moderate to well	165 (62.7)	98 (37.3)		177 (67.3)	86 (32.7)	
Lauren classification						
Diffuse	21 (67.7)	10 (32.3)	0.846	21 (67.7)	10 (32.3)	0.802
Intestinal	235 (63.9)	133 (36.1)		243 (66.0)	125 (34.0)	
Mixed	15 (68.2)	7 (31.8)		16 (72.7)	6 (27.3)	
TNM stage						
I-II	162 (67.5)	78 (32.5)	0.133	156 (65.0)	84 (35.0)	0.500
III-IV	110 (60.4)	72 (39.6)		124 (68.1)	58 (31.9)	
Distant metastasis						
M0	255 (65.1)	137 (34.9)	0.355	257 (65.6)	135 (34.4)	0.215
M1	17 (56.7)	13 (43.3)		23 (76.7)	7 (23.3)	
Chemotherapy						
XELOX	22 (75.9)	7 (24.1)	0.588	19 (65.5)	10 (34.5)	0.061
FOLFOX-4	50 (64.9)	27 (35.1)		43 (55.8)	34 (44.2)	
Others	21 (65.6)	11 (34.4)		18 (56.3)	14 (43.8)	
None	179 (63.0)	105 (37.0)		200 (70.4)	84 (29.6)	

the 3'UTR and coding regions of the *DNMT3a* and/or were shown that the polymorphism could alter the function of the gene in a biologically relevant manner according to the literature review; (iii) We selected SNPs across the *DNMT3a* gene loci to ensure a high representation of different blocks. As a result, two SNPs were selected among the candidate SNPs in DNMT3a. We focused on SNP in the promoter region (rs1550117 in promoter) and 3'UTR region (rs13420827) for genotyping.

Genotyping

Genomic DNA was extracted from whole blood sample using blood genomic DNA extraction kits following the manufacturer's instructions (Axygen Biosciences, Union City, USA). Genotypes of the selected SNPs were determined using the TaqMan discrimination assay in 384-well plates and read with the Sequence Detector Software V2.3 on the ABI PRISM 7900 HT Sequence Detector according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). Polymerase chain reaction (PCR) were carried out in a mixture containing 2 μl DNA (10 ng), 2.5 μl TagMan master mix, 0.25 μl SNP assay and 2.25 μl DNase-free H_2O (Applied Biosystems, Foster City, CA, USA). PCR conditions were as follows: 1 cycle of 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The amplification processes were performed on BIO-RAD S1000 thermal cyclers (Bio-Rad, California). Five percent of

Table 3. Association between *DNMT3a* genotypes and overall survival of gastric cancer

Genotypes	Patients	Deaths	MST	*P* value	Crude HR (95% CI)	Adjusted *P*[1]	Adjusted HR (95% CI)[1]
rs1550117							
GG	272	116	66.76		1.00		1.00
AG	136	71	35.63	0.035	1.37 (1.02-1.85)	0.052	1.34 (1.00-1.81)
AA	14	8	44.60	0.326	1.43 (0.70-2.93)	0.389	1.38 (0.67-2.85)
Dominant model							
GG	272	116	66.77		1.00		1.00
AG/AA	150	79	37.30	0.027	1.38 (1.04-1.84)	0.043	1.35 (1.01-1.80)
Recessive model							
GG/AG	408	187	66.77		1.00		1.00
AA	14	8	44.60	0.487	1.29 (0.63-2.61)	0.555	1.24 (0.61-2.55)
rs13420827							
CC	280	133	61.80		1.00		1.00
CG	124	55	46.33*	0.940	0.99 (0.72-1.35)	0.993	1.00 (0.72-1.38)
GG	18	7	50.02*	0.574	0.80 (0.38-1.72)	0.995	1.00 (0.46-2.15)
Dominant model							
CC	280	133	61.80		1.00		1.00
CG/GG	142	62	47.28*	0.807	0.96 (0.71-1.30)	0.992	1.00 (0.73-1.36)
Recessive model							
CC/CG	404	188	66.07		1.00		1.00
GG	18	7	50.02*	0.577	0.81 (0.38-1.72)	0.923	0.96 (0.45-2.06)

MST: median survival time; HR = hazards ratio; CI, confidence interval. *Mean OS was presented when median OS could not be calculated. [1]HRs and *P* values for each genotype were calculated adjusted for gender, age and clinical stage and chemotherapy type using Cox hazards model.

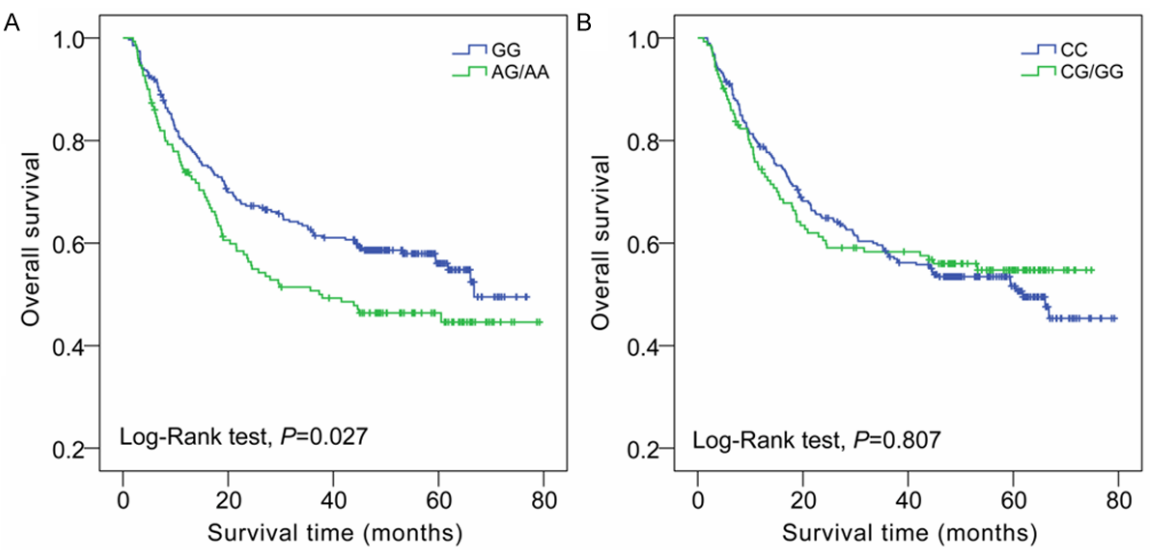

Figure 1. Association of *DNMT3a* genotypes with overall survival in gastric cancer patients. A. Plot for rs1550117 using the dominant model (AG/AA *vs*.GG). Patients carrying rs1550117 AG/AA genotype tended to live shorter than those carrying GG genotype, with a hazard ratio (HR) 1.38 (95% CI 1.04-1.84); B. Plot for rs13420827 using the dominant model (CG/GG vs. CC). No significance was found between rs13420827 polymorphism and overall survival of gastric cancer.

randomly-selected duplicate samples were included in each plate for quality control, and the overall concordant rate was >99% for all SNPs.

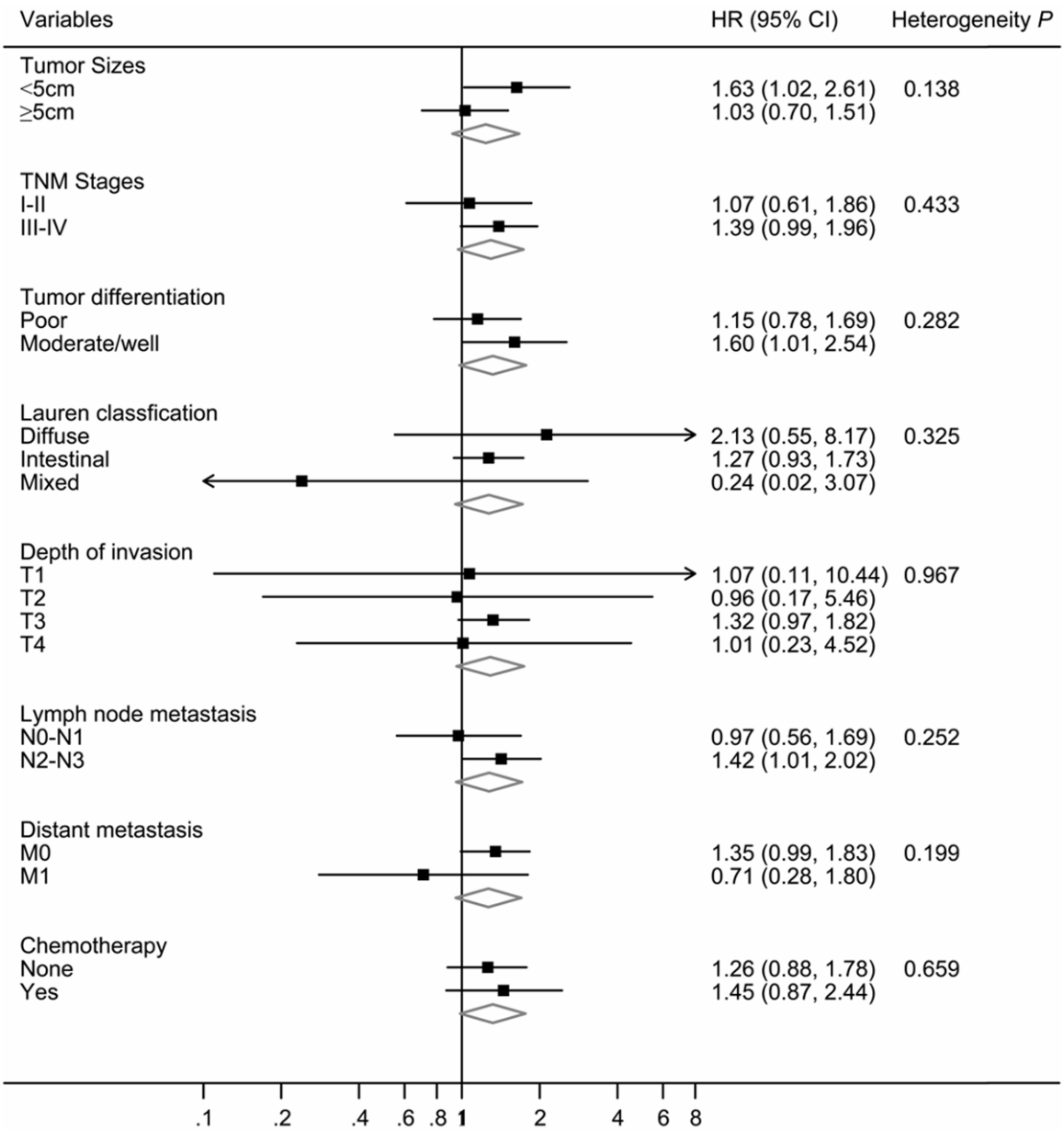

Figure 2. Stratified analysis for rs1550117 genotypes associated with gastric cancer patients' survival by genetic dominant model.

Statistical analysis

Survival functions of the gastric cancer patients within each SNP were plotted by Kaplan-Meier method and compared by Log-Rank test. Hazard ratio (HR) and their 95% confidential intervals (CIs) for OS were calculated by Cox proportional hazards regression model with adjustment for sex, age, tumor sizes and clinical stage. Multivariate Cox regression analysis was conducted to determine potential predictive factors of prognosis with a significance level of $P<0.05$ for entering and $P>0.10$ for removing the variables. Three genetic models (codominant, dominant and recessive) were used to assess their associations with the end point events. All test were two-sided and $P<0.05$ was considered statistically significant. All of the analyses were performed using SPSS-18.0 software (SPSS, Inc., Chicago, IL, USA).

Results

Characteristics of patients

A total of 447 patients with confirmed GC were enrolled in the study. Follow-up information was

Table 4. Stepwise Cox regression analysis of gastric cancer survival

Variables	HR	95% CI	*P*
Gender (Female vs. Male)	0.668	0.47-0.95	0.024
Tumor Sizes (≥5 cm vs. <5 cm)	1.746	1.30-2.35	<0.001
rs1550117 (AG/AA vs. GG)	1.375	1.03-1.84	0.032
TNM stages (III+IV vs. I+II)	5.557	4.01-7.70	<0.001

available for 435 (97.3%) of them until March 2015. Thirteen patients died of postoperative complications within 30 days at the beginning of the study and these cases were excluded from the analysis of effects of SNPs on survival. Finally, 422 patients were enrolled in the survival analysis. During the follow-up, 195 (46.2%) patients died from GC, 203 patients (48.1%) lived, 14 (3.3%) cases died of other disease and 10 (2.4%) patients were lost to follow up. The median follow-up time was 55.1 months with a range from 1.1 to 79.2 months (95% CI: 51.8-58.5). The clinicopathological data from the patients are shown in **Table 1**. Among these patients, intestinal histological type was the main diagnosis (368 patients, 87.2%), followed by 31 (7.4%) diffuse cancer, and the mixed-cell cancer (22 patients, 5.2%). We grouped the degree of differentiation into two categories: well and moderate or poor, and the number of the two groups were 155 and 263 (four cases with data missing), respectively. Of the 422 patients, 138 cases were received chemotherapy after surgery for at least 3 cycles and 284 cases were not. In detail, 77 patients received combined chemotherapy with 5-fluorouracil, leucovorin and oxaliplatin (FOLFOX-4 regimen), 29 cases received with capecitabine and oxaliplatin (XELOX regimen) and the remaining 32 patients received other chemotherapies such as leucovorin plus 5-fluorouracil (LV5-FU2) and capecitabine or 5-fluorouracil alone.

Clinical pathological characteristics including tumor sizes, TMN stages, depth of invasion, lymph node metastasis and distant metastasis were significantly associated with survival time of patients (log-rank $P<0.05$ for all). Patients with tumor sizes ≥5 cm, or with higher TNM stage, depth of invasion, lymph node metastasis and distant metastasis were at a dramatically higher risk of death compared to those with tumor size <5 cm, or with lower pathological stage, depth of invasion, lymph node metastasis and distant metastasis (log-rank $P<0.05$). However, tumor differentiation, histological type and chemotherapy type were not statistically significant associated with OS ($P=0.160$, 0.140 and 0.196, respectively).

Association of SNPs with clinicopathological parameters of gastric cancer

We further evaluated the association of *DNMT3a* rs1550117 and rs13420827 polymorphisms with clinicopathological parameters including histological type, tumor differentiation, TNM stage and distant metastasis in gastric cancer cases. However, no significant differences of the distribution frequency of *DNMT3a* polymorphisms were observed with the clinicopathological characteristics (**Table 2**).

Association between SNPs and survival of gastric cancer

We performed Cox proportional hazard models to assess the prognostic effect of rs1550117 and rs13420827 on GC patients in various genetic models. The results are shown in **Table 3**. We found that there was a significant association between the genotypes of rs1550117 and survival in the dominant model (**Figure 1A**). Patients carrying at least one variant allele (MST 37.30 months) had a shorter OS compared with the GG genotype (MST 66.77 months), suggesting a potential protective role of the wild G allele (HR=1.38, 95% CI: 1.04-1.84, $P=0.027$). After adjusting for sex, age, clinical stage and chemotherapy type, the rs1550117 A variant genotypes remained significantly associated with poor survival (HR=1.35, 95% CI: 1.01-1.80, $P=0.043$). However, we did not observe a significant association between rs13420827 polymorphism and overall survival of GC (dominant model: Log-Rank $P=0.807$) (**Figure 1B**). We further performed the stratified analysis for rs1550117 by tumor size, TNM stage, tumor differentiation, histology types, depth of invasion, lymph node metastasis, distant metastasis and chemotherapy. Compared with the GG genotype, the AG+AA variant genotype had a higher death risk in the subgroup of tumor sizes <5 cm, having moderate or well differential and with N2-N3 lymph node metastasis ($P<0.05$, **Figure 2**).

Cox regression analysis of gastric cancer survival

We performed multivariate stepwise Cox regression analysis to explore the independent prognostic factor for GC. Demographic characteristics, clinical features and rs1550117 polymorphism were selected as variables in the regression model. Finally, four predictive variables were included: gender (P=0.024), tumor sizes (P<0.001), clinical stage (P<0.001) and SNP rs1550117 (P=0.032). As shown in **Table 4**, the results also established rs1550117 SNP as an independent prognostic factor for poor survival of GC, patients carrying AG or AA variant genotypes had a 38% increased risk of death compared to those carrying GG genotype (HR=1.38, 95% CI: 1.03-1.84).

Discussion

In the present study, we for the first time investigated the effects of two SNPs (rs1550117 and rs13420827) of the *DNMT3a* gene on the progression and survival of gastric cancer in a Chinese population. We found that the rs1550117 AG/AA genotype was significantly associated with an increased rate of mortality compared to those carrying GG genotype. Furthermore, Cox regression analysis revealed that male, larger tumor sizes, higher TNM stages and variant genotype of rs1550117 remained independent adverse prognostic factors of gastric cancer.

The *DNMT3a* gene encodes DNMT3a, a member of DNA methyltransferase family, which is involved in a wide range of biological processes, including tumor development, differentiation and progression [29, 30]. Several studies have reported that genetic variant in Arg-to-His substitution at the coding region R882 (located in the methyltransferase domain) of *DNMT3a* gene are associated with acute myeloid leukemia prognosis. Patients with *DNMT3a* mutations had worse overall survival compared with *DNMT3a* wild-type patients [26, 31, 32]. The gene encoding *DNMT3a* is mutated in about 20% of acute myeloid leukemia cases, with Arg882 (R882) as the hotspot, which was apparently different from that in gastric cancer cases [33]. A possible explanation might be that the long-time existence of *DNMT3a*-R882H mutant leads to a significant increase of CDK1, and broken the relative balance of transcriptional controls between proliferation and differentiation in the cells to some extent. These results illustrated the function of *DNMT3a* mutations in myeloid leukemia. For this reason, the analysis of *DNMT3a* Arg882 mutation was not suitable as a marker for forecasting gastric cancer prognosis in our study.

As far as we know, most works published only focus on the role of *DNMT3a* in the carcinogenesis of malignancies. To our knowledge, this is the first report describing the association between *DNMT3a* polymorphism and gastric cancer prognosis. The rs1550117 SNP is the most frequently studied *DNMT3a* loci located in the promoter region, which is required for alteration the activity of the DNMT3a promoter [19]. Using bioinformatic tool (http://compbio.cs.queensu.ca/F-SNP/), rs1550117 is predicted to affect the transcriptional regulation of the DNMT3a mRNA, the G→A transition might increase the expression of DNMT3a, and increased expression of DNMT3a was associated with poor survival in gastric cancer. These suggest that DNMT3a is clinically useful for prediction of prognosis of gastric cancer. In prior studies, the rs1550117 SNP has been reported to contribute to susceptibility to colorectal cancer [20] and gastric cancer [19]. In contrast, we were unable to find the similar results for contributions of rs1550117 polymorphism to gastric cancer [34]. In this investigation, we found rs1550117 A allele was associated with a significantly increased poor survival of gastric cancer, suggesting that rs1550117 genetic variations may be employed as candidate biomarkers for the prediction of prognosis in Chinese GC patients. The effect of rs1550117 SNP may possibly be mediated through linkage to some other unknown key functional polymorphisms, and that linked SNP of *DNMT3a* may be conferring prognosis in our population. Due to lack of related research on other SNPs, whether this SNP have indirect role on gastric cancer survival required to be further elucidated.

A study by Yang et al. [11] detected rs13420827 in *DNMT3a* were significantly associated with GC susceptibility in the southern Chinese population. It was shown that the GC heterozygosity of rs13720827 to be protective effect for gastric cancer, with OR=0.66 (95% CI: 0.45-0.97, P=0.034). However, in our study, no positive association between rs13420827 poly-

morphism and gastric cancer survival could be observed. As no study was available on the rs13420827 and cancer survival, therefore, more studies may be needed to conclude the role of genetic variant at rs13420827 in the prognosis of gastric cancer.

Previous study has indicated that increased mRNA expression of three DNMTs were involved in the carcinogenesis, high expression levels of DNMT3a was associated with lower overall survival and poor prognosis [35-37]. Oh *et al.* [38] evaluated the mRNA levels of DNMT3a in 25 hepatocellular carcinomas (HCCs), and they found DNMT3a mRNA expression was associated with a >4-fold increase poorer recurrence-free survival in HCC tissues. And Yang *et al.* [24] found that DNMT3a expression was associated with TNM stage and lymph node metastasis in gastric cancer patients. In our previous study, expression of DNMT3a was also detected as an independent prognostic marker for GC [25]. These results revealed that increased DNMT3a expression may play an important role in tumor progression. However, the extensional mechanism requires further investigation.

Two limitations should be acknowledged in our study. First, although we observed a strong correlation between rs1550117 and overall survival of gastric cancer, how genetic variability at this locus influence *DNMT3a* through functional mechanism remain to be elucidated. Second, only two SNPs in *DNMT3a* are evaluated, it is possible that some other important SNPs are neglected. The observed associations may be due to other sites which are in linkage disequilibrium with the rs1550117. Therefore, larger well-designed studies are warranted to further assess the role of *DNMT3a* SNPs in the prognosis of gastric cancer in different polulations.

In conclusion, we found that *DNMT3a* functional SNP rs1550117 could serve as a significant biomarker to predict gastric cancer prognosis. Further investigations are needed to fully clarify the role of the *DNMT3a* on gastric cancer in different ethnic populations.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (No. 81373084; No. 81273065), Norman Bethune Program of Jilin University (No. 2013025), the Youth Foundation of The First Hospital of Jilin University (No. JDYY42013014; No. JDYY42013018) and Science and Technology Development Program of Jilin Province (20150414014GH).

Disclosure of conflict of interest

None.

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