RESEARCH COMMUNICATION

Genetic Variants of NBS1 Predict Clinical Outcome of Platinum-based Chemotherapy in Advanced Non-small Cell Lung Cancer in Chinese

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Abstract

Objective: NBS1 plays a key role in the repair of DNA double-strand break (DSB). We conducted this study to investigate the effect of two critical polymorphisms (rs1805794 and rs13312840) in NBS1 on treatment response and prognosis of advanced non-small cell lung cancer (NSCLC) patients with platinum-based chemotherapy. Methods: Using TaqMan methods, we genotyped the two polymorphisms in 147 NSCLC patients. Odds ratios (ORs) and their 95% confidential intervals (CIs) were calculated as a measure of difference in the response rate of platinum-based chemotherapy using logistic regression analysis. The Kaplan-Meier and log-rank tests were used to assess the differences in progression-free survival (PFS) and overall survival (OS). Cox proportional hazards model was applied to assess the hazard ratios (HRs) for PFS and OS. Results: Neither of the two polymorphisms was significantly associated with treatment response of platinum-based chemotherapy. However, patients carrying the rs1805794 CC variant genotype had a significantly improved PFS compared to those with GG genotype (16.0 vs. 8.0 months, P = 0.040). Multivariable cox regression analysis further showed that rs1805974 was a significantly favorable prognostic factor for PFS [CC/CG vs. GG: Adjusted HR = 0.62, 95% CI: 0.39-0.99; CC vs. CG/GG: Adjusted HR = 0.56, 95% CI: 0.32-0.97). Similarly, rs13312840 with a small sample size also showed a significant association with PFS (CC vs. CT/TT: Adjusted HR = 25.62, 95% CI: 1.53-428.39). Conclusions: Our findings suggest that NBS1 polymorphisms may be genetic biomarkers for NSCLC prognosis especially PFS with platinum-based chemotherapy in the Chinese population.

Keywords: Single-nucleotide polymorphism - NBS1 - NSCLC - chemotherapy -prognosis

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Introduction

Lung cancer is the most common cancer worldwide with approximately 1.5 million newly diagnosed cases and 1 million deaths annually (Le Chevalier, 2010). Non-small cell lung cancer (NSCLC) accounts for 75%-85% of lung cancer and most NSCLC patients are diagnosed in advanced stage (Spiro and Silvestri, 2005). Although platinum-based combination chemotherapy is the first-line treatment for advanced NSCLC patients, the response rate was only about 40% (Kelly et al., 2001; Schiller et al., 2002). The tumor, lymph node, metastasis (TNM) staging system of lung cancer is used widely for predicting prognosis (Naruke et al., 2001). However, differences in response rate and survival among NSCLC patients with the same stage and chemotherapy regimen suggest that other important factors, such as genetic background, may also affect the prognosis of individual patient. Therefore, great efforts have been made to evaluate the role of genetic polymorphisms in predicting therapeutic response and prognosis of NSCLC patients treated with chemotherapy.

Platinum-based drugs are cytotoxic through the formation of platinum-DNA cross-links and adducts, causing cell cycle arrest and ultimately apoptosis if not properly repaired (Siddik, 2003). The efficacy of each combination (platinum plus another agent) has been demonstrated to be similar by a series of trials in unselected patients with response rates of 30% to 40% (Scagliotti et al., 2002; Schiller et al., 2002; Ohe et al., 2007). It has been suggested that a major factor for the development of resistance to platinum-based drugs is the enhanced DNA repair capacity of tumor cells, which prevents the accumulation of lethal DNA damage from cytotoxic agents (Perez, 1998). Therefore, the differences in DNA repair capacity may play a role in the treatment response and prognosis of cancer patients

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after chemotherapy. In humans, DNA double-strand break (DSB) repair plays a critical role in maintaining genomic stability by two distinct mechanisms: nonhomologous end joining (NHEJ) and homologous recombination (HR) (Kobayashi et al., 2004). A protein complex MRN (consisting of MRE11, RAD50 and NBS1) has been identified as the most important component in both of these two DSB pathways (Maser et al., 1997). Among those, the NBS1 protein is the key regulator of the MRN complex, which recruits MRE11/RAD50 or retains them at the vicinity of DNA damage sites by direct binding to histone H2AX. Mutations in NBS1 significantly downregulate MRN complex expression and markedly disrupt its function, thus enhancing cisplatin-induced DNA damage and cytotoxicity (Tran et al., 2004; Araki et al., 2010). Studies in mice have indicated that heterozygous for NBS1-null mutations develop tumors in lung, liver, mammary gland and prostate (Dumon-Jones et al., 2003; Zhang et al., 2006). NBS1 overexpression has been observed in several smoking-related cancer sites including NSCLC, hepatoma, esophageal cancer and head and neck squamous cell carcinoma (HNSCC) (Chen, 2005; Yang, 2006). Furthermore, it was also correlated with recurrence/metastasis of oral squamous cell carcinoma and the poor prognosis of HNSCC (Yang, 2006; Hsu et al., 2010). Thus, NBS1 may play an important role in the development and prognosis of human cancers.

The NBS1 gene encompasses 48797 bps of genomic sequence on chromosome 8q21 with about 48 single nucleotide polymorphisms (SNPs) according to the National Center for Biotechnology Information dbSNPs public databases (Zhang et al., 2006). Rs1805794 located in exon 5 of NBS1 is one of the most common polymorphisms and can induce an amino acid substitution from Glu to Gln. Studies have investigated the association between rs1805794 and risk of multiple cancers, such as lung, breast, bladder and nasopharyngeal carcinoma (Medina et al., 2003; Lan et al., 2005; Lu et al., 2006; Hsu et al., 2007; Stern et al., 2009; Zheng et al., 2011). However, only one study has explored the effect of this polymorphism on prognosis of human cancer and reported that it was not associated with the survival of esophagus or gastric adenocarcinoma after platinum-based neoadjuvant poly-chemotherapy (Ott et al., 2011). Besides, rs13312840 (T>C) is another potentially functional SNP in NBS1, which is located in the 5' near gene of NBS1 and may modify the expression levels of NBS1 gene by interfere its' binding with transcription factor GATA-1 (Merika and Orkin, 1993). Studies have showed that rs13312840 was significantly associated with the risk of breast cancer in European and Cypriot populations (Lu et al., 2006; Loizidou et al., 2010). However, no study has focused on the association between rs13312840 and cancer prognosis. Given the important role of NBS1 in DSB repair, we hypothesize that the two SNPs in NBS1 may affect the function of NBS1 and then have associations with treatment response and prognosis of advanced NSCLC patients with platinum-based chemotherapy.

To validate this hypothesis, we investigated the association between these two polymorphisms of NBS1 (rs1805794 and rs13312840) and clinical outcome of lung cancer in 147 advanced NSCLC patients treated with platinum-based chemotherapy.

Materials and Methods

Study populations

All patients in the study were recruited from the First Affiliated Hospital of Nanjing Medical University (Jiangsu, China) between January 2004 and September 2010. All cases were newly diagnosed, histopathologically confirmed and without prior history of other cancers or previous chemo- or radio- therapy. Only inoperable, advanced NSCLC (IIIB–IV) patients were included in the study to avoid the potential confounding effect from surgery and clinical stage. Patients were interviewed face-to-face to collect demographic data including age, sex and smoking status. Those who had a low smoking frequency (<1 cigarette per day) and duration (<1 year) in their lifetime were defined as non-smokers; otherwise, they were classified as smokers. Each patient donated 5-ml venous blood after written informed consent was obtained. Finally, a total of 147 advanced NSCLC patients with available blood sample were included in our study. Patients' response to platinum-based (cisplatin or carboplatin) regimen was assessed after the first two or three cycles and determined by the Response Evaluation Criteria in Solid Tumors (RECIST) criteria 1.1 (Eisenhauer et al., 2009). All responses were re-evaluated at least 4 weeks after initial assessment. For data analysis, complete response (CR) and partial response (PR) were combined as responders, and stable disease (SD) along with progressive disease (PD) were grouped as non-responders. Follow-up was performed per three months from the time of enrollment till death or the latest follow-up. Progression-free survival (PFS) was defined as the time from first treatment to the date of disease progression (PD), death or last follow-up. Overall survival (OS) was calculated as the time between first dose and death or the last follows.

DNA collection and genotyping

Genomic DNA was extracted from a leukocyte pellet by traditional proteinase K digestion and followed by phenol-chloroform extraction and ethanol precipitation. SNPs (rs1805794 and rs13312840) in NBS1 were genotyped with TaqMan genotyping assays, using the ABI 7900 real-time PCR system (Applied Biosystems

Table 1. Information for Primers and Probes by TaqMan Allelic Discrimination

Polymorphism	Sequence (5'-3')
rs1805794	
Primer	F : GACGTCCAATTGTAAAGCCAGA
	R : CCTTTCAATTTGTGGAGGCTG
Probe	FAM-CTGAAAGCAGTTGAGTC-MGB
	HEX-CTGAAAGCAGTTCAGTC-MGB
rs13312840	
Primer	F : CACTCTAGCCTGGGCGATAGA
	R : CTAATATTGTGCTTAGGAGTTTGCATCT
Probe	FAM-TACCAATCTCTTATCATC-MGB
	HEX-TACCAATCTCTTGTCATC-MGB

Table 2. Patient Characteristics and Clinical Features

Characteristics	Response				PFS			OS			
	Patients N (%)	Responders N (%)	P[a]	Progression N (%)	MST (months)	HR(95% CI)	Log-rank P	Death N (%)	MST (months)	HR(95% CI)	Log-rank P
Age (years)											
<=60	74(50.3)	26(35.1)	0.815	47(63.5)	7.0	1	0.162	21(28.4)	33.0	1	0.729
>60	73(49.7)	27(37.0)		43(58.9)	12.0	0.74(0.48-1.13)		28(38.4)	32.0	1.11(0.62-1.98)	
Gender											
Male	92(62.6)	34(37.0)	0.768	57(62.0)	9.0	1	0.304	35(38.0)	31.0	1	0.030
Female	55(37.4)	19(34.6)		33(60.0)	11.0	0.79(0.50-1.24)		14(25.5)	33.0	0.50(0.26-0.95)	
Smoking status[b]											
Never	79(54.1)	34(43.0)	0.042	43(54.4)	13.0	1	0.008	18(22.8)	33.0	1	<0.001
Ever	67(45.9)	18(26.9)		46(68.7)	6.0	1.80(1.16-2.78)		30(44.8)	36.0	2.94(1.61-5.40)	
Histology type											
Squamous cell	39(26.5)	15(38.5)	0.231	24(61.5)	11.5	1	0.861	15(38.5)	13.1[d]	1	
Adenocarcinoma	100(68.0)	33(33.0)		62(62.0)	9.0	0.96(0.59-1.56)		32(32.0)	33.0	0.61(0.32-1.14)	0.264
Others[c]	8(5.4)	5(62.5)		4(50.0)	5.3[d]	0.96(0.80-1.15)		2(25.0)	16.4[d]	0.90(0.70-1.15)	
Clinical Stage											
III	21(14.3)	6(28.6)	0.441	11(52.4)	11.5	1	0.584	5(23.8)	11.7[d]	1	0.588
IV	126(85.7)	47(37.3)		79(62.7)	9.0	1.20(0.63-2.29)		44(34.9)	32.0	1.29(0.51-3.29)	

HR, hazard ratio; CI, confidence interval; MST, median survival time; PFS, progression-free survival; OS, overall survival; [a]P value for $\chi 2$ test; [b]Information of smoking status was available in 146 cases; [c]Other carcinomas include large cell, undifferentiated and mixed-cell carcinoma; [d]Mean survival time was provided when MST could not be calculated

Inc., Foster City, CA). The sequences of primers and probes were listed in Table 1. SDS allelic discrimination software (version 2.3, provided by ABI) was used for analysis of genotyping results. All the genotyping assays were performed with two blank (water) controls for quality control in each 384-well format and more than 10% samples were randomly selected to repeat, yielding a 100% concordant. The success rates of genotyping for rs1805794 and rs13312840 were 95.9% and 98.6%, respectively.

Statistical analysis

Hardy-Weinberg equilibrium was assessed by a goodness-of-fit χ^2 test. Odds ratios (ORs) and their 95% confidential intervals (CIs) were calculated as a measure of difference in the response rate using logistic regression analysis (responders vs. non-responders). The Kaplan-Meier and log-rank tests were used to assess the differences in PFS and OS. Median survival time (MST) was calculated, and mean survival time was presented when the MST could not be obtained. Cox proportional hazards model was applied to assess the hazard ratios (HRs) for PFS and OS. The adjustment factors included age, gender, smoking status, histology and stage. The statistical analyses were performed using Statistical Analysis System software (version 9.1.3, SAS Institute, Cary, NC). All P-values were two-sided, and P-value <0.05 was considered statistically significant.

Results

Patient characteristics and clinical features

The demographic characteristics and clinical information for the 147 NSCLC patients recruited in the study were summarized in Table 2. The median age was 60 years (range, 32-82 years). Of the 147 NSCLC patients, 92 (62.59%) were male and 67 (45.89%) were smokers. Among these patients, 100 (68.03%) were adenocarcinoma, 39 (26.53%) were squamous cell carcinomas and the others (8 patients, 5.44%) were large cell, undifferentiated and mixed-cell carcinomas. All the patients had advanced inoperable lung cancer, with 14.29% of stage IIIB and 85.71% of stage IV. The median PFS and OS for the 147 patients were 10.0 months (range, 1.0-60.0 months) and 33.0 months (range, 1.5-81.7 months), respectively. Smoking was an unfavorable factor for response rate (P = 0.042), PFS (HR = 1.80, 95% CI: 1.16-2.78) and OS (HR =2.94, 95% CI: 1.61-5.40). However, other clinical parameters were not associated with outcome of NSCLC patients.

Table 3. Treatment Characteristics of the Enrolled Patients

Chemotherapy regimens	Patients (N%)
DDP/CBP + TAX/TXT/DOC	92 (62.6)
DDP/CBP + GEM	36 (24.5)
DDP/CBP + NVB	13 (8.8)
DDP/CBP + Pemetrexed	6 (4.1)

DDP, cisplatin; CBP, carboplatin; TAX, taxol/paclitaxel; TXT, tanetere; DOC, docetaxel; GEM, gemcitabine; NVB, vinorelbine

Polymorphisms and treatment response

All patients had received platinum-based chemotherapy: 92 received TP/TC (DDP/CBP plus taxol/taxotere/docetaxel), 36 had GP/GC regimens (DDP/CBP plus gemcitabine), 13 had NP/NC (DDP/CBP plus vinorelbine), and 6 were given DDP/CBP plus pemetrexed regimens (Table 3). The concrete dosage were as follows: DDP 75 mg/m^2 on Day 1; CBP area under the curve (AUC) 5-6 g on Day 1; taxol 175 mg/m^2 on Day 1 (kept for 3h); taxotere 75 mg/m^2 on Day 1 (kept for 1h); docetaxel 60 mg/m^2 on Day 1 (kept for 1h); gemcitabine 1250 mg/m^2 on Days 1 and 8; vinorelbine 25 mg/m^2 on Days 1 and 8; pemetrexed 800 mg/m^2 on Day 1. All the chemotherapeutic agents were administered intravenously. Treatment cycles were repeated every 3-4 weeks, for 3-6 cycles, unless unacceptable toxicity or disease progression appeared.

Overall, 53 (36.1%) patients demonstrated responders,

Table 4. Genotyping of SNPs in NBS1 and their Associations with Chemotherapy Response, PFS and OS

Genotype	Patients N (%)	Response responders N (%)	Response Adjusted OR (95% CI)[a]	PFS Progression N (%)	PFS MST (months)	PFS Adjusted HR (95% CI)[a]	PFS Log-rank P	OS Deaths N (%)	OS MST (months)	OS Adjusted HR (95% CI)[a]	OS Log-rank P
rs1805794											
GG	47(33.3)	15(31.9)	1	32(68.1)	8.0	1	1	19(40.4)	25.5	1	1
CG	59(41.9)	23(38.9)	1.46(0.60-3.56)	38(64.4)	9.0	0.74(0.44-1.22)	0.435	19(32.2)	36.0	0.61(0.31-1.20)	0.494
CC	35(24.8)	14(40.00)	1.46(0.55-3.86)	18(51.4)	16.0	0.43(0.23-0.81)	0.040	10(28.6)	33.0	0.46(0.20-1.06)	0.326
CC/CG vs. GG	94(66.7)	37(39.4)	1.46(0.66-3.21)	56(63.6)	--	0.62(0.39-0.99)	0.133	29(60.4)	--	0.57(0.31-1.04)	0.330
CC vs. CG/GG	106(75.2)	38(35.9)	1.31(0.57-3.04)	70(79.6)	--	0.56(0.32-0.97)	0.092	38(79.2)	--	0.70(0.34-1.47)	0.511
Additive model			1.26(0.78-2.05)			0.69(0.51-0.93)	0.161			0.71(0.47-1.06)	0.597
rs13312840											
TT	108(74.5)	39(36.1)	1	66(61.1)		1	1	38(35.2)		1	1
TC	36(24.8)	13(36.1)	0.94(0.41-2.16)	22(61.1)	9.0	0.76(0.46-1.27)	0.363	10(27.8)	31.0	0.59(0.28-1.22)	0.259
CC	1(0.7)	0(0.0)	--	1(100.0)	13.0	24.6(1.36-446.1)	0.006	1(100.0)	28.3[b]	--	<0.001
CC/TC vs. TT	37(25.5)	13(35.1)	0.90(0.39-2.06)	23(25.8)	1.5	0.80(0.48-1.32)	0.461	11(22.5)	1.5	0.66(0.33-1.33)	0.381
CC vs. TC/TT	144(99.3)	52(36.1)	--	88(98.9)	--	25.6(1.53-428.4)	0.003	48(98.0)	--	--	--
Additive model			0.86(0.39-1.91)			0.85(0.52-1.40)	0.009			0.75(0.38-1.48)	--

OR, odds ratio; HR, hazard ratio; CI, confidence interval; MST, median survival time; PFS, progression-free survival; OS, overall survival; [a]Adjusted for age, gender, smoking status, histology and stage; [b]Mean survival time was provided when MST could not be calculated

Table 5. Stratified analysis of rs1805794 genotypes associated with PFS of NSCLC patients

Variable	CC (Progression/Patients)	CG/GG (Progression/Patients)	Adjusted HR(95% CI)	P[b]
Age (years)				
<=60	12/21	35/52	0.56 (0.26-1.20)	0.952
>60	6/14	35/54	0.54 (0.22-1.33)	
Gender				
Male	10/21	45/66	0.48 (0.23-1.02)	0.403
Female	8/14	25/40	0.78 (0.33-1.84)	
Smoking status				
Never	9/19	34/59	0.65 (0.30-1.42)	0.795
Ever	8/15	36/47	0.56 (0.25-1.26)	
Histology type				
Squamous cell carcinoma	6/13	16/23	0.26 (0.09-0.79)	0.144
Adenocarcinoma	12/21	50/77	0.68 (0.34-1.36)	
Others[a]	0/1	4/6	--	
Clinical Stage				
III	3/5	8/13	0.77 (0.15-3.95)	0.675
IV	15/30	62/93	0.53 (0.29-0.97)	

HR, hazard ratio; CI, confidence interval; [a]Other carcinomas include large cell, undifferentiated and mixed-cell carcinoma; [b]P-value for the heterogeneity test

94 (63.9%) showed no-responders. The distributions of genotypes were shown in Table 4. Genotype frequencies are in agreement with those expected according to the Hardy-Weinberg equilibrium model (P = 0.062 and 0.277 for rs1805794 and rs13312840, respectively). However, neither of the two polymorphisms of NBS1 was significantly associated with treatment response.

Polymorphisms and survival

Log-rank tests showed that NSCLC patients carrying rs1805794 CC genotype had an improved PFS compared to those with GG genotype (16 vs. 8 months, P = 0.040). However, rs13312840 CC genotype significantly decreased PFS compared to TT genotype (1.5 vs. 9 months, P = 0.006).

Similarly, multivariate cox regression analysis revealed a significant effect of NBS1 rs1805794 on patients' PFS after the adjustment for age, gender, smoking status, histology type and clinical stage (CC/CG vs. GG: adjusted HR = 0.62, 95% CI: 0.39-0.99; CC vs. CG/GG: adjusted HR = 0.56, 95% CI: 0.32-0.97) (Table 4, Figure 1). Additionally, the variant homozygous of rs13312840 with a small sample size also showed a significant association with PFS of NSCLC (CC vs. TC/TT: HR = 25.62, 95% CI: 1.53-428.39). While only one subject was rs13312840 CC. Ultimately, the results should be treated with caution. No such associations were detected between these two polymorphisms and OS of NSCLC.

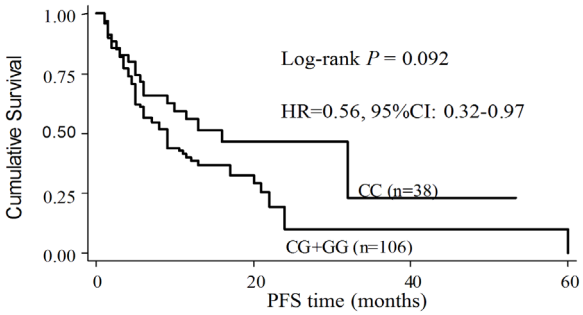

Figure 1. Kaplan-Meier Plot of PFS in Relation to the NBS1 rs1805794 Genotypes. HR, hazard ratio; CI, confidence interval; PFS, progression-free survival

The associations between NBS1 rs1805794 polymorphism and PFS of advanced NSCLC were further stratified by age, gender, smoking, histology and stage. As shown in Table 5, the association remained significant among subjects with squamous cell carcinoma (HR = 0.26, 95% CI: 0.09-0.79) and clinical stage IV (HR = 0.53, 95% CI: 0.29-0.97). No significant heterogeneity existed in every stratum.

Discussion

This study demonstrated that NBS1 rs1805794 and rs13312840 variant genotypes were significantly associated with prognosis especially PFS in NSCLC patients with platinum-based chemotherapy, indicating that genetic variants in NBS1 might play a role in modulating advanced NSCLC patients' prognosis.

The NBS1 protein has three known functional regions: the N-terminus (amino acids 1-183), a central region (amino acids 278-343), and the C-terminus (amino

acids 665-693) (Kobayashi et al., 2004). Among those, the N-terminal region contains a fork-head associated (FHA) domain and two breast cancer carboxy-terminal (BRCT) domains. Rs1805794 is a missense polymorphism (Glu185Gln) located in the BRCT domain of NBS1 (Kobayashi, 2004), and such a domain facilitates NBS1 to interact with breast cancer type 1 susceptibility protein (BRCA1) and form a BRCA1-associated genome surveillance complex (BASC) which is responsible for recognition and repair of aberrant DNA (Wang et al., 2000; Futaki and Liu, 2001). Thus, the Glu185Gln may interfere with protein-protein interaction and affect DNA repair capacity (Tauchi, 2000). Rs1061302, high linkage disequilibrium (LD) with rs1805794 (D'=1, R2=1 in CHB), was located in the MRN complex binding site and considered to be associated with increased risk of smoking-related cancers including lung cancer by Park et al. SNPs in LD with rs1061302 may inadvertently affect proper binding of the MRN complex and thus alter its ability to repair or detect DNA DSB (Park et al., 2010). Thus, we hypothesized that the C allele of rs1805794 confers a low level of MRN complex compared to the wild-type. Taken together, it seems this C variant allele is associated with decreased DNA repair activity. DNA repair has been termed a double-edged sword, with the reason that although decreased DNA repair may increase the susceptibility of cancer, it might also simultaneously improve survival in patients already diagnosed with cancer, when treated with agents inducing DNA damage like cisplatin or carboplatin (Li et al., 2009; Wang et al., 2011).

Publications on NBS1 polymorphisms have mainly focused on rs1805794 and the risk of cancers including lung, breast, bladder, renal cell cancers, non-Hodgkin lymphoma and childhood acute leukemia (Medina et al., 2003; Lan et al., 2005; Zienolddiny, 2005; Landi et al., 2006; Ryk et al., 2006; Hsu et al., 2007; Choudhury et al., 2008; Margulis et al., 2008; Mosor et al., 2008; Schuetz et al., 2009; Stern et al., 2009; Park et al., 2010), but the results were not consistent. A recent meta-analysis on rs1805794 has shed light on a positive association between the variant genotype of rs1805794 and the risk of multiple cancers (MeiXia et al., 2009). However, few studies have investigated the relationship between NBS1 and cancer patients' prognosis (Yang, 2006; Hsu et al., 2010; Ott et al., 2011). The only one study enrolling 258 esophageal cancer and gastric adenocarcinoma patients who receiving platin/5-Fu/leucovorin-based poly-chemotherapy showed that rs1805794 in NBS1 was not associated with recurrence or OS of patients with any of these two cancers in European population (Ott et al., 2011). Interestingly, we found that the NBS1 rs1805794 variant genotypes were associated with a longer PFS and OS, although the latter is not statistically significant. Different ethnicities, tumor sites or clinical characteristics of patients may explain why we observed associations while the previous study did not. Our findings supported that the variant allele of rs1805794 may affect DNA repair activity and in turn, play a role in the prognosis of NSCLC patients treated with platinum-based chemotherapy. The exact mechanisms for such association still need to be explored by functional studies.

Rs13312840 is located in the 5' UTR of NBS1 gene which is the binding site of transcription factor GATA-1 (Merika and Orkin, 1993). However, few studies have reported the association of this SNP with cancer development except that two studies focused on its effect on breast cancer risk with inconsistent results (Lu et al., 2006; Loizidou et al., 2010). In our study, we found a significant association between the homozygous variant genotype and an unfavorable prognosis; however, low power may be induced by small sample size of variant genotype. Therefore, our findings should be interpreted with caution and larger sample studies are needed to further understand this SNP.

Although our study reported some new findings about polymorphisms of NBS1 in NSCLC patients with chemotherapy, several potential limitations should be taken into consideration. Firstly, we only included two common functional SNPs, which is far from comprehensive. The observed associations may result from other polymorphisms in LD with the studied ones. Secondly, our study lacked the related phenotypic and functional assays, which limited our inquiry into the functional consequence of these variants. Moreover, our sample size was relatively small and other larger studies would address the associations of more SNPs with chemotherapy response and prognosis in such a patient population.

In summary, our findings suggest that polymorphisms in NBS1 may be genetic markers for prognosis of advanced NSCLC patients receiving platinum-based chemotherapy, which need the further validation from larger patient cohorts and functional evaluations.

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