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Research Paper

Polymorphisms of *CCNB1* Associated With the Clinical Outcomes of Platinum-Based Chemotherapy in Chinese NSCLC Patients

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Abstract

As a crucial cell cycle regulator and G2/M phase promotor, *CCNB1* played an essential role in progression of chemotherapy related cell death. Platinum-based chemotherapy is still the first-line chemotherapy regimen for most advanced NSCLC patients. We aim to investigate the correlation of *CCNB1* polymorphisms to the efficiency of platinum-based chemotherapy in Chinese advanced NSCLC patients. We enrolled 972 patients with advanced NSCLC, and extracted DNA from their peripheral blood for genotyping *CCNB1* four tagSNPs which selected from the Hapmap database. We analyzed the association of *CCNB1* four tagSNPs with efficiency of platinum-based chemotherapy. We found that rs2069429 and rs2069433 of *CCNB1* were associated with the OS of advanced NSCLC patients. Patients with GG genotype of rs2069429 had longer OS than non-GG patients (HR=0.81, 95%CI=0.68-0.95, p=0.009); and patients with AA genotype of rs2069433 had longer OS than non-AA patients (HR=0.78, 95%CI=0.61-0.98, p=0.036). And the haplotype GAAA of *CCNB1* was a putative factor in subgroup patients with clinical stage IV. The association of *CCNB1* polymorphisms and toxicities after platinum-based chemotherapy was assessed. Rs2069433 and rs350104 were related with gastrointestinal toxicity of platinum-based chemotherapy. The patients with GG genotype of rs2069433 (p=0.013) and/or non-GG genotype of rs350104 (p=0.042) may have a severe gastrointestinal toxicity after chemotherapy, and then clinician may can reduce the dosage of chemotherapy agents to avoid sever toxicities in these patients. In summary, *CCNB1* polymorphisms may contribute to the clinical efficiency of platinum-based chemotherapy in advanced NSCLC patients, and it is helpful for the personalized treatment.

Key words: Platinum, Chemotherapy, CCNB1, Single nucleotide polymorphism, Lung cancer.

Introduction

With high morbidity and mortality, 48.3/10^5 and 39.3/10^5, respectively, lung cancer is a leading cause of cancer related death disease in the worldwide, including China [1]. Lung cancers are divided into two subtypes according to histology, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC is account for 85% in whole lung cancer patients. No matter what the histology of lung cancer is, the patients are diagnosed in advanced clinical stag (stage III or IV) at the first time, with losing chance of surgical resection [2]. And then chemotherapy, targeted therapy, and immune therapy are becoming promising treatment for advanced lung cancer patients. However, the patients harboring positive driver mutation are only accounting for merely 10% and 2%–7%, respectively (for EGFR driver mutation and ALK rearrangement). Based on 2015 NCCN guidelines for NSCLC, the

patients without typical mutations still rely on chemotherapy, and the platinum-based double chemotherapy is the first-line chemotherapy regimen in advanced lung cancer patients [3].

The efficiency of chemotherapy is not identical among patients and this individual differences may can be explained by single nucleotide polymorphisms (SNP), a single base differences among people in human genome which amount to approximately 15 million [4]. To better understanding the links between disease and genome, many databases about SNP were created by sequencing, like the international HapMap project, 1000 Genomes Project. Many research have been reported lung cancer susceptibility was affected by genome variants at different populations [5-8]. To find the dominantly polymorphisms of some gene which can predict the clinical outcomes of cancer are essential for patients with NSCLC.

As a cell cycle regulator, CCNB1 (also called cyclin B1), combined with CDK1 to be a complex, is a crucial factor for cell cycle, controlling the cell cycle G2/M phase [9]. The tyrosine 15 site phosphorylated of CCNB1-CDK1 can make cells entry into mitosis in normal status, leading to cell proliferation. The activation of CCNB1-CDK1 complex contributes to switch-like all or none behavior which decide to devote to mitosis. It is a positive feedback loop make sure that the activated CCNB1-CDK1 complex is perpetual. CCNB1 was reported with overexpressed in many cancers [10-12] (such as lung cancer, breast cancer, gastric and so on), and associated with chemotherapy resistance germ cell tumors [13]. Research has been reported that *CCNB1* polymorphisms were critical for disease, not only for cancers. For example, Silvestre-Roig *et al.* found that the genetic variants of *CCNB1* rs350099, rs350104, and rs164390 increased the risk of coronary in-stent restenosis [14]. We can see that *CCNB1* was played an important role in diseases' formation. CCNB1 was also treated as a tumor antigen which holds promise as a putative cancer vaccine [15]. A part of functions and features of *CCNB1* were listed in Table S1. We aim to investigate the relationship between *CCNB1* tagSNPs/haplotypes and efficiency of platinum-based chemotherapy in advanced lung cancer, we enrolled 972 advanced NSCLC patients treated with platinum-based double regimen in our study and sequenced their tagSNPs (rs352626, rs2069429, rs2069433, and rs350104), which choose from the hapmap database based on criteria: 1) minor allele frequency (MAF) cutoff equals 0.05 and 2) correlation coefficient (r2) threshold equals 0.8 (details see **Material and Methods**), and then analyzed their association using log-rank test, univariate and multivariate cox's regression analysis. In our study, we found that *CCNB1* polymorphisms contribute to NSCLC patients' clinical benefits.

Material and Methods

Ethical approval

The procedures involving human beings performed in our study were complying with ethical standards of research committee in Shanghai Pulmonary Hospital, Shanghai Changhai Hospital, Shanghai Chest Hospital, and Shanghai Zhongshan Hospital. It is in accordance with 1964 Helsinki declaration and its later amendments ethical standards. We informed all the advanced NSCLC patients in this study and got their written consent form. No animals were used for research in our study by any of authors. All experiments involving in our study were performed under approved guidelines and regulations.

Study cohorts

Our study recruited 972 patients with advanced (stage III or IV) NSCLC who were treated by first-line platinum-based double chemotherapy regimen from March 2005 to February 2010. All the patients received surgery, radiotherapy were excluded when enrolled. Whole patients' Eastern Cooperative Oncology Group performance status (ECOG PS) were in 0-2 points. The chemotherapy toxicities were assessed with hematologic, cardiovascular, hepatic and gastrointestinal. The association of *CCNB1* tagSNPs with clinical outcomes in advanced NSCLC patients treated by platinum-based chemotherapy was evaluated.

Treatment

972 advanced NSCLC patients in our study were received platinum-based chemotherapy regimen, including double-DNA damage agents (cisplatin or carboplatin combined gemcitabine), platinum-tubulin targeting agents (cisplatin or carboplatin combined navebline, paclitaxel or docetaxel), and other combination (cisplatin or carboplatin combined etoposide or bevacizumab). They accepted above regimen for two to six cycles. The dosage for cisplatin and carboplatin was 75 mg/m^2 and AUC 5 respectively on day 1 every three weeks. 1250 mg/m^2 on days 1 and 8 every three weeks was for gemcitabine. The usages for tubulin-targeting agents, navelbine, paclitaxel and docetaxel were 25 mg/m^2 on days 1 and 8 every three weeks, 175 mg/m^2 on day 1 every three weeks, 75 mg/m^2 on day 1 every three weeks respectively.

Clinical outcomes

Clinical outcomes after platinum-based

chemotherapy with advanced NSCLC patients contained overall survival (OS), progression-free survival (PFS), disease control rate (DCR), and objective response rate (ORR) were evaluated by Response Evaluation Criteria in Solid Tumors. OS was predominantly for evaluating the efficacy of chemotherapy, started from the first day of receiving chemotherapy to death or to the final follow-up in our study. PFS reflecting the short-term response of chemotherapy, was defined from the day accepted chemotherapy to disease progression or the day of death (whichever occurred first) or the last follow-up for progression-free. DCR was consist with complete response (CR), partial responses (PR), and stable disease (SD). ORR was comprised of CR and PR.

Toxicity

Chemotherapy toxicities were evaluated twice weekly based on the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. We collected the worst toxic records at the beginning two cycles of therapy for analysis. Toxicities in our study dominantly assessed hematologic (neutropenia, thrombocytopenia, and anemia), cardiovascular, hepatic and gastrointestinal (vomiting, nausea). Grades 3 or 4 was defined to severe toxic effects; grade 5 meant death. However, no grade 5 was observed in this study.

CCNB1 tagSNPs and haplotypes

Four tagSNPs of *CCNB1*, (rs352626, rs2069429, rs2069433 and 350104) were selected from CHB data of the phase 2 HapMap SNP database (http://www.hapmap.org/) for study. Then we extracted the patients' DNA from their peripheral blood using QIAamp DNA Maxi Kit (Qiagen GmbH, Hilden, Germany). All the experiments were according to the manufacturer's guidelines for genotyping purpose. We genotyped the patients' tagSNPs with iSelect HD Bead-Chip (Illumina, San Diego, CA, USA), and the quality-control criteria are following: 1) Minor allele frequency (MAF) > 0.05; 2) Genotyping call rate > 0.95, and 3) GenCall score > 0.2 (Figure S1). The concordance of replicates was over 99.9%. The personal haplotype frequencies of *CCNB1* four tagSNPs were calculated by PHASE 2.0 Program (version 2.0.2) based on the Bayesian algorithm (Table S2).

Statistical analysis

We did statistical analysis with the SPSS version 20.0 software (SPSS INc. Chicago, IL, USA). The Kaplan-Meier curves and univariate Cox regression analysis were used for illustrating relationship between patients' OS/PFS and four tagSNPs of *CCNB1*. The independent significant factors were adjusted by multivariate Cox proportional hazard model, including clinical factors and *CCNB1* tagSNPs related to OS/PFS of advanced NSCLC patients. For studying the relationship between the significant tagSNPs of *CCNB1* and DCR or CCR, we used X^2 test. Correlation between *CCNB1* tagSNPs/haplotypes and severe toxicities was assessed by univariate logistic analysis. All statistical analysis considered the differences of $p < 0.05$ as statistically significant and defined two sides.

Results

Study Patients and Patients' clinical characteristics

The study patients were recruited from Shanghai Changhai Hospital, Shanghai Zhongshan Hospital, Shanghai Chest Hospital and Shanghai pulmonary Hospital. Our study included 972 patients, which consisted with 690 male patients and 282 female patients. The patients' clinical characteristics were involving age, sex, smoking status, clinical stage, histology of tumor, ECOG PS, and chemotherapy regimen. The detail of the patients' clinical characteristics and OS/PFS were listed in the Table 1. All patients were diagnosed with advanced NSCLC, which clinical stage was III or IV. The patients' median age was 57.8 years old. The youngest patient was 26 years old in this study, and patients with 82 years were the oldest. For tumor histology, we classified it with three types, adenocarcinoma, squamous carcinoma, and others (included adenosquamous carcinoma, mixed-cell, neuroendocrine, or undifferentiated carcinoma). The patients with adenocarcinoma type were most than the other types, account for 63.4 percent in 972 patients. Every patient in our study received platinum-based chemotherapy regimen. About 70.9 percent of the total patients, accepted the platinum-tubulin-targeting regimen chemotherapy. Patients who received the double DNA damage agent chemotherapy accounted for 24.3 percent. The rest patients used other combination regimen. Then we analyzed the association between clinical characteristics and patients' OS/PFS using log-rank test (Table 1).

We found that age, sex, smoking status, and tumor histology of the clinical characteristics were associated with patients' OS. For patients' age, the younger patients (<60 years) had longer OS than older one (>=60 years) (X^2=8.95, p=0.003). Female patients may had a beneficial survival time when compared with the male patients (X^2=8.87, p=0.003). Patients who smoke a lot had shorter OS than non-smoking patients (X^2=6.30, p=0.012). Patients with lung

adenocarcinoma can be survive longer than other histology's patients (X^2=6.40, p=0.04). The other clinical characteristics, such as clinical stage, ECOG PS, and chemotherapy regimen, were not significantly related to the patients' OS. Only one clinical characteristic, ECOG PS, was significantly associated with PFS (p=0.001). No differences were observed when analyzed relationship between other clinical factors and PFS (Table 1).

CCNB1 rs2069429 and rs2069433 were associated with OS

We have chosen four tagSNPs (rs352626, rs2069429, rs2069433, and rs350104) of *CCNB1* from the HapMap database, which followed some criteria (see **Material and Methods**). The genotype frequencies and MAF (minor allele frequency) of *CCNB1* four tagSNPs were calculated among the current data or Han Chinese in Beijing, China (CHB), Utah residents with Northern and Western European ancestry from the CEPH collection (CEU), and Yoruban in Ibadan, Nigeria (YRI). The data of CHB, CEU, and YRI population were collected from the NCBI website (https://www.ncbi.nlm.nih.gov/). The results were shown in table 2. The MAF of *CCNB1* four tagSNPs in our study were close to the MAF of CHB, CEU population from HapMap data (data not show). Our MAF of *CCNB1* can be represented for the CHB and CEU population in some extent, not for the YRI population.

Then the association between *CCNB1* tagSNPs and OS/PFS was analyzed by log-rank test, univariate and multivariate COX' regression analysis. The four tagSNPs of *CCNB1* were classified to three types, genotype, and dominant/recessive model. We find that rs2069429 genotype and rs2069433 dominant (G) model were significantly associated with OS through univariate analysis. The patients with GG genotype (mOS=19.5, 95%CI=17.9-21.2) of rs2069429 had longer OS than patients with AG genotype (mOS=17.9, 95%CI=15.5-20.3) of rs2069429 (AG *vs.* GG, HR=1.2, 95%CI=1.02-1.41, p=0.025) (Figure 1). Another tagSNP, rs2069433, was associated with patients' OS too. Patients with AA genotype of rs2069433 had longer months (3.2 month) than non-AA genotype's patients (HR=0.75, 95%CI=0.60-0.94) (Figure 1, Table 3). However, the other tagSNPs, rs352626, rs350104 were not correlated with OS of NSCLC patients through univariate analysis.

Table 1. The clinical characteristics of 972 advanced NSCLC patients treated with platinum-based chemotherapy.

Variables	N =972	OS			PFS		
		mOS (95%CI)	X^2	$P_{l\text{-}g}$	mPFS(95%CI)	X^2	$P_{l\text{-}g}$
Median age, y (range)	57.8(26-82)						
<60	502(51.8%)	21.3(19.3-23.4)	8.95	3E-3*	7.7(6.2-9.2)	0.249	0.618
≥60	467(48.2%)	17.2(15.3-19.0)			9.5(8.0-11.0)		
Censored	3						
Sex							
Male	690(71.0%)	18.1(16.5-19.6)	8.87	3E-3*	9.4(8.0-10.8)	1.322	0.250
Female	282(29.0%)	22.5(19.0-26.0)			7.7(5.8-9.5)		
Smoking status							
Nonsmoker	405(41.8%)	21.2(19.0-23.4)	6.30	0.012*	7.8(6.3-9.3)	0.614	0.433
Ever Smoker	563 (58.2%)	17.9(16.1-19.7)			9.5(7.9-11.1)		
Censored	4						
Clinical stage							
IIIa	76 (7.9%)	23.0(14.6-31.)	4.40	0.111	12.6(6.9-18.3)	4.371	0.112
IIIb	283(29.3%)	19.1(17.1-21.1)			9.8(7.2-12.4)		
IV	608(62.8%)	19.1(16.9-21.2)			8.0(6.8-9.2)		
Censored	1						
ECOG PS							
0-1	879(91.7%)	19.4(18.0-20.8)	3.078	0.079	9.3(8.1-10.6)	10.775	0.001*
2	80(8.3%)	17.9(9.6-26.2)			5.4(3.4-7.5)		
Censored	3						
Tumor histology						0.230	0.891
adenocarcinoma	612(63.4%)	20.2(18.4-22.1)	6.40	0.040*	9.1(7.7-10.6)		
Squamous carcinoma	213(22.0%)	16.6(13.1-20.2)			9.5(6.8-12.1)		
others[a]	141(14.6%)	18.3(14.8-21.8)			8.0(6.2-9.8)		
Censored	6						
Doublet regimen						5.119	0.077
DNA damaging agents	236(24.3%)	19.8(17.2-22.3)	1.95	0.378	9.3(7.6-11.0)		
Platinum-tubulin-targeting drugs[b]	689(70.9%)	19.1(17.3-20.9)			9.1(7.6-10.7)		
Other combination[c]	47(4.8%)	20.2(15.6-24.8)			5.1(2.7-7.6)		

[a] Others NSCLC included adenosquamous carcinoma, mixed-cell, neuroendocrine carcinoma, or undifferentiated carcinoma.
[b] Tubulin-targeting drugs includes paclitaxel, docetaxel or navelbine.
[c] Other combination included etoposide or bevacizumab.
*p<0.05. Abbreviations: mOS: median overall survival, CI: confidence interval

Table 2. Genotype frequencies and MAF of *CCNB1* tag SNPs in current data, or in CHB, CEU and YRI from HapMap SNP database.

Tag SNPs	Location	Allele frequencies				
		Current Data	CHB	CEU	YRI	*p*
Rs352626	5′-near gene					
MAF		0.470(A)	0.463(A)	0.447 (A)	0.456(G)	0.016*
A/A		207(21.3%)	16(19.5%)	44(19.5%)	68(30.1%)	
A/G		499(51.3%)	44(53.7%)	114(50.4%)	110(48.7%)	
G/G		266(27.4%)	22(26.8%)	68(30.1%)	48(21.2%)	
Rs350104	intron					
MAF		0.313(G)	0.367(G)	0.392(G)	0.158(G)	<0.0001
A/A		462(47.5%)	32(35.6%)	42(35.0%)	82(68.3%)	
A/G		412(42.4%)	50(55.6%)	62(51.7%)	38(31.7%)	
G/G		98(10.1%)	8(8.89%)	16(13.3%)	0	
Rs2069429	5′-near gene					
MAF		0.180 (A)	0. 233(A)	NA	NA	<0.0001
A/A		31(3.2%)	0(0%)	0	0	
A/G		287(29.5%)	42(46.7%)	0	0	
G/G		654(67.3%)	48(53.3%)	120(100%)	120(100%)	
Rs2069433	intron					
MAF		0.055(G)	0.056(G)	0.083(G)	0.192(G)	<0.0001
A/A		869(89.4%)	80(88.9%)	100(83.3%)	76(63.3%)	
A/G		100(10.3%)	10(11.1%)	20(16.7%)	42(35.0%)	
G/G		3(0.3%)	0	0	2(1.7%)	

*p<0.05. Abbreviations: UTR, untranslated region; MAF, Minor allele frequency; CHB, Han Chinese in Beijing, China; CEU, Utah residents with Northern and Western European ancestry from the CEPH collection; YRI, Yoruban in Ibadan, Nigeria

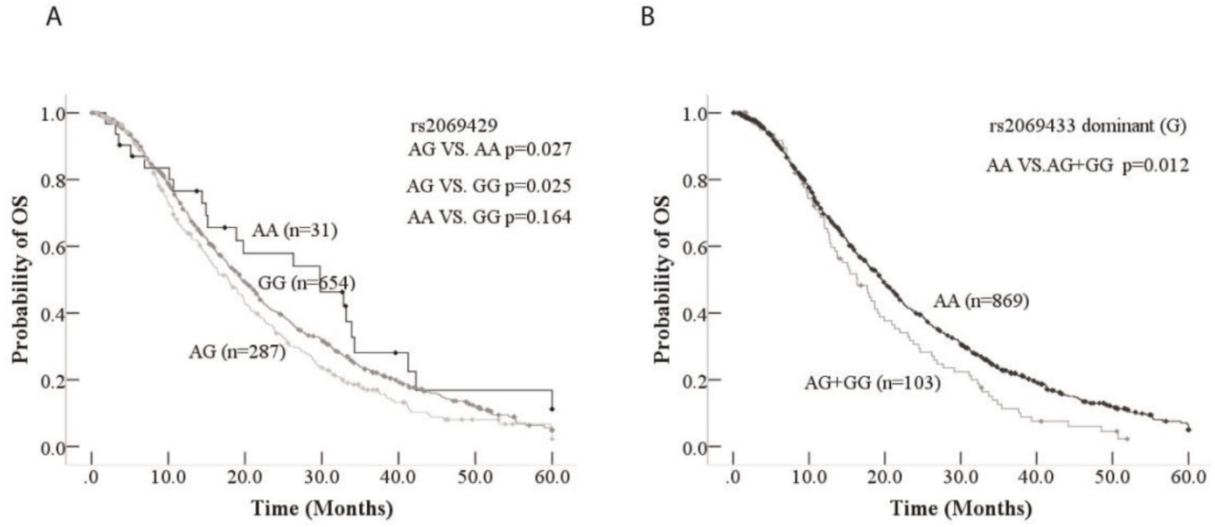

Figure 1. Kaplan-Meier curve of association between rs2069429/rs2069433 of *CCNB1* and OS in advanced NSCLC patients. A: Kaplan-Meier curve of rs2069429 of *CCNB1* to advanced NSCLC patients' OS **B:** Kaplan-Meier curve of rs2069433 of *CCNB1* to advanced NSCLC patients' OS

The clinical factors, such as age, sex, TNM, ECOG PS, smoking, histology, treatment, and four tagSNPs were included into multivariate cox's regression analysis. For clinical characteristics, age and sex were significantly associated with advanced NSCLC patients' OS according to multivariate cox's regression analysis (Table 4). Younger patients (<60 years old) obviously had longer OS than older (>=60) (HR=1.23, 95%CI=1.06-1.43, p=0.006); Female had longer OS than male (HR=0.81, 95%CI=0.69-0.96, p=0.015). Among four tagSNPs, rs2069429 dominant (A) and rs2069433 dominant (G) were significantly observed association with OS based on multivariate cox's regression analysis. Patients with GG genotype of rs2069429 had longer OS than non-GG patients (HR=0.81, 95%CI=0.68-0.95, p=0.009); and patients with AA genotype of rs2069433 had longer OS than non-AA patients (HR=0.78, 95%CI=0.61-0.98, p=0.036) (Table 4). Above all, in term of *CCNB1* tagSNPs, rs2069429 dominant (A) and rs2069433 dominant (G) were independent predictive factors of advanced NSCLC patients' OS in our study.

When PFS was used for endpoint, only ECOG PS was significantly associated with patients' PFS according to multivariate cox's regression analysis (p=0.003) (Table S4). However, no significantly tagSNPs were observed through univariate and multivariate analysis (Table S3, Table S4).

Table 3. Univariate analysis of four tagSNPs of *CCNB1* with OS in 972 advanced NSCLC patients.

SNP ID	genotype	N(%)	mOS(95%CI)	X^2	$P_{L\text{-}G}$	HR(95%CI)	P_{COX}
Rs352626				0.952	0.621		0.622
Genotype	AG	499(51.3%)	19.1(17.1-21.0)			R	
	GG	266(27.4%)	19.8(17.1-22.6)			0.92(0.77-1.09)	0.338
	AA	207(21.3%)	19.3(16.0-22.2)			0.99(0.82-1.19)	0.892
Dominant(A)	AA +AG	706(72.6%)	19.1(17.3-20.8)				
	GG	266(27.4%)	19.8(17.1-22.6)	0.933	0.334	0.92(0.78-1.09)	0.335
Recessive(A)	AA	207(21.3%)	19.1(15.9-22.2)				
	GG+AG	765(78.7%)	19.3(17.7-20.8)	0.036	0.850	0.98(0.83-1.17)	0.850
Rs2069429				7.92	0.019*		0.020*
Genotype	GG	654(67.3%)	19.5(17.9-21.2)			R	
	AG	287(29.5%)	17.9(15.5-20.3)			1.20(1.02-1.41)	0.025*
	AA	31(3.2%)	29.8(14.3-45.3)			0.73(0.48-1.13)	0.157
Dominant(A)	AA+AG	318(32.7%)	18.2(16.0-20.5)				
	GG	654(67.3%)	19.5(1.9-21.2)	2.58	0.108	0.88(0.76-1.03)	0.109
Recessive(A)	AA	31(3.2%)	29.8(14.3-45.3)				
	GG+AG	914(96.8%)	19.1(17.7-20.5)	2.81	0.094	1.44(0.94-2.20)	0.096
Rs2069433				7.52	0.023*		0.025*
Genotype	A/A	869(89.4%)	19.6(18.2-21.1)			R	-
	A/G	100(10.3%)	16.4(13.0-19.8)			1.32(1.05-1.66)	0.019
	G/G	3(0.3%)	12.9(10.4-15.4)			2.3(0.74-7.17)	0.151
Recessive(G)	AA+AG	969(99.7%)	19.3(17.9-20.6)	2.02	0.155	0.45(0.14-1.40)	0.166
	GG	3(0.3%)	12.9(10.4-15.4)				
Dominant(G)	AA	869(89.4%)	19.6(18.2-21.1)	6.33	0.012	0.75(0.60-0.94)	0.012*
	GG+AG	103(11.6%)	16.4(12.9-20.0)				
Rs350104				1.14	0.566		0.567
genotype	A/A	462(47.5%)	18.8(17.0-20.6)			R	-
	A/G	412(42.4%)	19.7(17.6-21.7)			0.93(0.80-1.08)	0.343
	G/G	98(10.1%)	20.3(16.2-24.5)			0.91(0.71-1.17)	0.461
Recessive(G)	AA +AG	874(90.9%)	19.1(17.7-20.5)	0.23	0.631	1.06(0.84-1.35)	0.632
	GG	98(10.1%)	20.3(16.2-24.5)				
Dominant(G)	AA	462(47.5%)	18.8(17.0-20.6)	1.12	0.291	1.08(0.94-1.25)	0.292
	GG+AG	510(53.5%)	19.8(18.0-21.7)				

*p<0.05. Abbreviations: mOS: median overall survival, HR: hazard ratio, CI: confidence interval, NA: Not available, R: Reference

CCNB1 haplotype GAAA was related with OS in patients with clinical stage IV

We analyzed the relationship between tagSNPs of *CCNB1* and the OS/PFS, and we found rs2069429 dominant (A) and rs2069433 dominant (G) were valuable tagSNPs for advanced NSCLC patients. We explored the haplotype of this four tagSNPs using PHASE 2.0 software and analyzed the association between haplotype of *CCNB1* and OS/PFS. Eight haplotypes based on the rs352626, rs350104, rs2069429, rs2069423 were calculated by PHASE 2.0 program. We analyzed three haplotypes which the frequencies were more than 10%. Haplotype GAAA (haplotype 1), haplotype GGGA (haplotype 4), haplotype AAGA (haplotype 7) frequencies were 18%, 31.3%, 41.5% respectively (Table S3). Then we analyzed this three haplotypes and the OS/PFS using univariate and multivariate analysis. No significantly association was observed between three haplotypes and OS/PFS (Table S5). However, stratified analysis according to TNM clinical stag shows that haplotype GAAA was a predictable factor of patients' OS. Patients who was in clinical stage IV with zero copy number GAAA had 3.6 months longer OS than one or two copy number GAAA's patients (p=0.021,Table 5, Figure 2). And stage IV patients with haplotype GGGA one or two copy number was slightly significant than zero copy number's patients (p=0.051, Table 5). This haplotype GAAA may connected with lung cancer metastatic, resulting to the significantly differences in patients with clinical stag IV rather than stage III. However, we did not find any haplotype can be a predictable factor for patients' PFS under different clinical stage (Table S6).

CCNB1 tagSNPs/haplotypes association with toxicities after chemotherapy

Excepting survival time, there were other important factors that may influence patients' treatment efficiency, which was the side effects of treatment. And then we analyzed the links between four tagSNPs, three haplotypes of *CCNB1* and toxicities after platinum-based chemotherapy using univariate logistic regression analysis. The results displayed in Table 6, rs2069433 recessive (G) model and rs350104 recessive (G) model were related with the gastrointestinal toxicity after platinum-based chemotherapy. The patients with GG genotype of rs2069433 (p=0.013) and/or non-GG genotype of

rs350104 (p=0.042) may have a severe gastrointestinal toxicity after chemotherapy. If the patients were with GG genotype of rs2069433 or non-GG genotype of rs350104, the clinicians can reduce the dosage of platinum and other chemotherapy agents for the patients to avoid sever gastrointestinal toxicities. No haplotypes of *CCNB1* four tagSNPs were related with the toxicities in our study by analysis (data not shown).

Discussion

Many studies have been reported that genes' SNP were related with the efficiency of platinum-based chemotherapy in lung cancer, including our previous studies about SNP [16]. Nowadays, many targeted drugs such as EGFR tyrosine kinase inhibitors (TKIs [erlotinib, gefitinib, and afatinib]) is widely used in NSCLC patients, however, the number of patients with EGFR driver mutation is limited, only 17% , and only 7% of lung adenocarcinoma patients having ALK translocation (also driver mutation gene)[17]. For the rest large number of advanced NSCLC patients, chemotherapy remains the first-line treatment, especially platinum-based double regimen. Much research has reported that genes are related to efficacy of chemotherapy drugs, such as WEE1, and ERCC1 for platinum and gemcitabine, respectively, and TUBB3 for paclitaxel [16, 18, 19]. *CCNB1* as a critical M-phase promoting factor in cell cycle, may be a next putative gene to predict efficiency of platinum-based chemotherapy for advanced NSCLC patients according to our study. This is the first time that we investigate the association between *CCNB1* four tagSNPs/haplotypes (rs352626, rs350104, rs2069429, rs2069433) and OS/PFS in advanced NSCLC patients treated with platinum-based chemotherapy. The results showed that rs2069429 dominant (A) and rs2069433 dominant (G) of *CCNB1* were significantly associated with OS of NSCLC patients who treated with platinum-based chemotherapy. The GG genotype of rs2069429 and AA genotype of rs2069433 were positive factors for advanced NSCLC patients, following with longer OS. Our finding is consistent with Li et.al. 's research, they have been reported rs2069429 recessive model of *CCNB1* was associated with breast cancer susceptibility, progression and survival, with increasing breast cancer risk [20]. Ma and colleagues studied the relation between 21 cell cycle genes (including *CCNB1*) and survival time of 586 Chinese NSCLC patients, they also find *CCNB1* rs2069429 was associated with the survival time of patients in univariate analysis [21]. We explored the rs2069429 function in online SNP function predictive software (https://snpinfo.niehs.nih.gov/cgi-bin/snpinfo/snpfunc.cgi). Rs2069429 is located in the 5'-near gene, it belongs to transcription factor binding site (TFBS), which means an important role in *CCNB1* gene expression. Rs2069429 may can control the cell-cycle progression by regulating the expression of CCNB1, and then resulting to affect the efficiency of chemotherapy, or even survival time of NSCLC patients. However, the function of *CCNB1* intron rs2069433 is not yet clear. Rs2069433 may influence OS of advanced NSCLC patients through linkage with rs2069429, which can mediate CCNB1 expression indirectly.

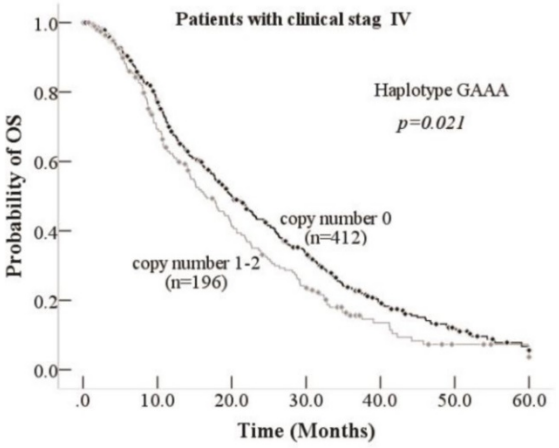

Figure 2. Kaplan-Meier curve of association between Haplotype GAAA and OS inpatients with clinical stage IV

Table 4. Multivariate Cox's regression analysis of clinical factors and *CCNB1* tagSNPs for overall survival in the 972 advanced NSCLC patients treated with platinum-based chemotherapy.

Variables	HR (95% CI)	p
Age (>=60 *vs.*<60)	1.23(1.06-1.43)	0.006*
Sex (male *vs.* female)	0.81(0.69-0.96)	0.015*
TNM(IV *vs.* IIIa *vs.* IIIb)	1.11(0.99-1.24)	0.076
ECOG PS(2 *vs.*1)	1.21(0.92-1.57)	0.171
Smoking (ever *vs.* never)	1.04(0.84-1.29)	0.741
Histology		0.181
adenocarcinoma	R	
squamous	1.19(0.97-1.46)	0.096
adenosquamous	1.29(0.74-2.24)	0.378
others	1.22(0.97-1.54)	0.087
Treatment		0.568
DNA-damaging agents [a]	R	
Platinum-tubulin-targeting drugs [b]	0.96(0.80-1.15)	0.632
Other combination [c]	0.83(0.57-1.20)	0.321
Rs2069429 dominant (A)	0.81(0.68-0.95)	0.009*
Rs2069429 recessive (A)	1.54(0.98-2.41)	0.063
Rs2069433 dominant (G)	0.78(0.61-0.98)	0.036*
Rs2069433 recessive (G)	0.69(0.16-2.89)	0.607
Rs350104 dominant (G)	0.96(0.79-1.17)	0.702
Rs350104 recessive (G)	0.94(0.73-1.21)	0.641
Rs352626 dominant (A)	0.92(0.72-1.81)	0.517
Rs352626 recessive (A)	0.88(0.61-1.27)	0.498

a Others NSCLC included adenosquamous carcinoma, mixed-cell, neuroendocrine carcinoma, or undifferentiated carcinoma.

b Tubulin-targeting drugs includes paclitaxel, docetaxel or navelbine.

c Other combination included etoposide or bevacizumab.

*p<0.05. Abbreviations: HR: hazard ratio, CI: confidence interval;

Table 5. Stratified analysis of association between *CCNB1* haplotypes with OS in patients with clinical stage III and IV

Haplotype	Patients with clinical stage III				Patients with clinical stage IV			
	N (%)	mOS(95% CI), mo	X^2	*P*-value	N (%)	mOS(95% CI), mo	X^2	*P*-value
GAAA								
Copy number 0	238(66.3%)	19.3(17.0-21.5)	-	0.814	412(67.8%)	20.0(17.4-22.6)	-	0.021*
Copy number 1-2	121 (33.7%)	19.8 (16.2-23.4)	0.055		196(32.2%)	16.4(13.2-19.6)	5.304	
GGGA								
Copy number 0	167 (46.5%)	19.8(17.2-22.4)	-	0.424	292(48.0%)	18.0(15.8-20.1)	-	0.051
Copy number 1-2	192(53.5%)	19.3(17.5-21.1)	0.640		316(52.0%)	20.2(17.3-21.1)	3.814	
AAGA								
Copy number 0	121(33.7%)	20.4(17.5-23.3)	-	0.296	201(33.1%)	17.7(15.0-20.4)	-	0.770
Copy number 1-2	238(66.3%)	19.1(16.1-22.0)	1.093		407(66.9%)	19.5(17.4-21.6)	0.085	

*p<0.05. Abbreviations: mOS: median overall survival; mo: months, HR: hazard ratio, CI: confidence interval

Table 6. Association between *CCNB 1* tagSNPs/haplotypes and toxicities in the 972 advanced NSCLC

Toxicities	SNP/haplotype	grade3/4(%)	OR(95%CI)	*p*
Gastrointestinal	Rs2069433 recessive (G)			
	GG	2/3(66.7%)	-	
	AA+AG	74/933(7.9%)	0.05(0.00-0.53)	0.013*
	Rs350104 recessive (G)			
	GG	2/95(2.1%)	-	
	AA+AG	74/841(8.8%)	4.37(1.06-18.2)	0.042*

*p<0.05. Abbreviations: OR: odds ratio, CI: confidence interval

The haplotypes of these four tagSNPs (rs352626, rs350104, rs2069429, rs2069423) were analyzed by PHASE 2.0 software program. Three haplotypes, GAAA, GGGA, and AAGA, which frequencies are over 10%, were chosen for study. No one was significant with OS in whole patients after univariate and multivariate analysis. However, when we stratified with TNM clinical stag, haplotype GAAA was a valuable factor for clinical stag IV patients. For clinical stag IV patients, harboring zero copy number GAAA has 3.6 months longer OS than patients with one or two copy number. This haplotype GAAA may related with NSCLC's metastatic, leading to the significantly differences in patients with clinical stag IV rather than stage III. We can find scientists have been reported that CCNB1 expression is associated with metastasis and tumor differentiation degree of hypopharyngeal squamous cell carcinoma [22]. We can infer that the haplotype of GAAA may be related with CCNB1 expression, because of rs2069429's TFBS function. No significantly association between *CCNB1* tagSNPs/haplotypes and patients' PFS. We can see the similar results in treatment studies when drugs can affect immune function. A possible reason why *CCNB1* polymorphisms influence OS but not PFS may be that the treatment drugs' role of pharmacogenetics can altered the metabolism, even the immune function [23, 24]. CCNB1 is transiently expressed in the cell cycle, however, CCNB1 was identified to be a new tumor antigen in year of 1997 and then found CCNB1 was overexpressed in many cancers, such as lung, breast, and colorectal cancer and involved in the CD8+ T cell immune progression [25, 26, 27, 28]. The correlation between *CCNB1* and the immune system may be the reason why polymorphisms of *CCNB1* is associated with OS, but not PFS in our study.

Due to resistance and toxicities of drugs, the 5-year overall survival time of lung cancer patients is 16.1% in China over last 40 years [1]. Toxicities of chemotherapy drugs effect patients' quality life, even survival time. Many researches have reported that polymorphisms can be a predictive factor for patients' toxicities. Qian reported that polymorphisms of *Caspase 8* and *Caspase 10* was related with advanced NSCLC patients' hematologic toxicity in treatment of platinum-based chemotherapy [29]. The association between *CCNB1* tagSNPs/haplotypes and toxicities was assessed in our study. We found that rs2069433 recessive (G) model and rs350104 recessive (G) model were putative factor for gastrointestinal toxicity. We may infer that patients harboring GG genotype of rs2069433 and/or non-GG genotype of rs350104 have a server gastrointestinal toxicity than others. The clinicians can regulate the dosage of chemotherapy drugs in clinical treatment to avoid sever toxicities, and then increase patients' quality of life, even survival time.

In summary, *CCNB1* is a crucial cell-cycle regulator and G2/M phase promotor in cell's proliferation and differentiation. Only one article investigated the polymorphisms of *CCNB1* in NSCLC before us. They found rs2069429 of *CCNB1* was associated with the survival time of patients in univariate analysis, however, no significant *CCNB1* SNPs associated with survival time in multivariate analysis, which may can explain with limited patients [21]. We enrolled 972 patients in our study and first come up with that rs2069429 and rs2069433 of *CCNB1*

were associated with the OS of advanced NSCLC patients. Patients holding GG genotype of rs2069429 had longer OS than non-GG patients (p=0.009); and patients with AA genotype of rs2069433 had longer OS than non-AA patients (p=0.036). We also accessed the haplotype of *CCNB1* four tagSNPs and clinical outcomes in 972 advanced NSCLC patients. The haplotype GAAA of *CCNB1* was a potential factor in subgroup patients with clinical stage IV in our study. The contribution of *CCNB1* SNPs in toxicities after platinum-based chemotherapy were evaluated as well. Rs2069433 and rs350104 were correlated with gastrointestinal toxicity after receiving platinum-based chemotherapy. If validated, rs2069429 and rs2069433 of *CCNB1* can be a putative biomarker for NSCLC patients treated with platinum-based chemotherapy. Haplotype GAAA of *CCNB1* was predictive for clinical IV stage subgroup patients largely. The polymorphisms of *CCNB1* were related with patients' toxicities after treatment. All in all, *CCNB1* polymorphisms may contribute to the clinical outcomes of platinum-based chemotherapy in advanced NSCLC. We still need further prospective studies of *CCNB1* polymorphisms.

Supplementary Material

Supplementary figures and tables.
http://www.jcancer.org/v08p3785s1.pdf

Abbreviations

CCNB1: cyclin B1
OS: overall survival
PFS: progression-free survival
NSCLC: non-small-cell lung cancer
ALK: anaplastic lymphoma kinase
EGFR: epidermal growth factor receptor
NCCN: National comprehensive cancer network
SNP: Single nucleotide polymorphisms
CHB: Han Chinese in Beijing
CEU: Utah residents with Northern and Western European ancestry from the CEPH collection
YRI: Yoruban in Ibadan
TKIs: tyrosine kinase inhibitors
ECOG PS: Eastern Cooperative Oncology Group performance status
RECIST: Response Evaluation Criteria in Solid Tumors
DCR: disease control rate
CR: complete response
PR: partial response
SD: stable disease
NCI: National Cancer Institute
CTCAE: Common Terminology Criteria for Adverse Events

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Competing Interests

The authors have declared that no competing interest exists.

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