RESEARCH PAPER

Cancer Biology & Therapy 15:11, 1542–1551; November 2014; © 2014 Taylor & Francis Group, LLC

Polymorphisms of p53 and MDM2 genes are associated with severe toxicities in patients with non-small cell lung cancer

Datong Zheng[1,2], Yanping Chen[2], Caijie Gao[2], Yongyue Wei[2], Guochun Cao[3], Nan Lu[4], Yayi Hou[5], Xiuqin Jiang[2,*], and Jianjun Wang[1,*]

[1]Department of Biological Science and Technology and State Key Laboratory of Pharmaceutical Biotechnology; School of Life Sciences; Nanjing University; Nanjing, China; [2]The Research Center; Second Hospital; Nanjing Medical University; Nanjing, China; [3]Nanjing Medical University Affiliated Cancer Hospital; Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research; Nanjing, China; [4]School of Clinical Medicine; Shanghai Medical College; Fudan University; Shanghai, China; [5]Immunology and Reproductive Biology Lab & Jiangsu Key Laboratory of Molecular Medicine; Medical School; Nanjing University; Nanjing, China

Keywords: MDM2, non-small cell lung cancer, p53, polymorphism, toxicity

Abbreviations: CBR, clinical benefit rate; CR, complete response; NSCLC, non-small cell lung cancer; ORR, objective response rate; PCR-RFLP, PCR-based restriction fragment length polymorphism; PD, progressive disease; PR, partial response; PS, performance status; SCLC, small-cell lung cancer; SD, stable disease; SNP, single nucleotide polymorphism; TNM, tumor/node/metastasis.

Adverse events in platinum-based chemotherapy for patients with advanced non-small cell lung cancer (NSCLC) are major challenges. In this study, we investigated the role of the p53 and MDM2 genes in predicting adverse events in NSCLC patients treated with platinum-based chemotherapy. Specifically, we examined the p53 p. Pro72Arg (rs1042522), MDM2 c.14 + 309T>G (rs2279744) and MDM2 c.− 461C > G (rs937282) polymorphisms using PCR-based restriction fragment length polymorphism (RFLP) in 444 NSCLC patients. We determine that MDM2 c.14 + 309T > G was significantly associated with severe hematologic and overall toxicities for advanced NSCLC patients treated with platinum-based chemotherapy, especially for patients aged 57 and younger. This was also true for patients with adenocarcinoma. Second, we determine that severe gastrointestinal toxicities in patients with heterozygous MDM2 c.−461C > G were significantly higher than in patients with the G/G genotype. Third, patients with the MDM2 c.−461C > G − c.14 + 309T > G CT haplotype show much higher toxicities than those of CG haplotype. Moreover, patients carrying the MDM2 c.−461 > G –c.14 + 309T > G CG/CT diplotype exhibited higher toxicities than those carrying CG/CG. Fourth, we found that the p53 p. Pro72Arg polymorphism interacts with both age and genotype. In addition, no significant associations were observed between the 3 SNPs and the response to first-line platinum-based chemotherapy in advanced NSCLC patients. In summary, we found that the p53 p. Pro72Arg, MDM2 c.14 + 309T > G and MDM2 c.−461C > G polymorphisms are associated with toxicity risks following platinum-based chemotherapy treatment in advanced NSCLC patients. We suggest that MDM2 c.14 + 309T > G may be used as a candidate biomarker to predict adverse events in advanced NSCLC patients who had platinum-based chemotherapy treatment.

Introduction

Lung cancer continues to be a serious global health issue and is one of the leading causes of cancer-related death worldwide, of which non-small cell lung cancer (NSCLC) accounts for over 80%.[1] Nearly 2-thirds of NSCLC patients are diagnosed at late or advanced stages, the majority of them at stage III or IV.[1] Chemotherapy can prolong the survival of advanced NSCLC patients, with platinum-based chemotherapy regimens being the standard first-line therapy for advanced disease. However, the treatment-related toxicities, including hematologic and gastrointestinal toxicities, remain major challenges in treatment of patients with advanced NSCLC.[2,3]

Chemotherapeutic drugs cause various types of DNA damage, including DNA adducts and double/single-strand breaks, and kill cancer cells mainly via apoptotic pathways.[4] A growing number of studies suggest that individual variations in treatment, clinical outcome and toxicities of platinum-based combination chemotherapy for lung cancer are associated with gene polymorphisms, which in turn affect drug-metabolizing, drug-transporting and DNA repair enzymes.[5–8] Here, we chose to focus on the MDM2 polymorphisms in the pathway of p53-mediated apoptosis in patients treated with platinum-based combination chemotherapy.

The p53 tumor suppressor gene encodes a short-lived transcription factor that is the principal mediator of multiple cellular

*Correspondence to: Xiuqin Jiang; Email: jiangxiuq@gmail.com; Jian-Jun Wang; Email: jjwang@nju.edu.cn
Submitted: 01/10/2014; Revised: 05/14/2014; Accepted: 08/16/2014
http://dx.doi.org/10.4161/15384047.2014.956599

functions. It is activated by cellular stimuli such as genotoxic stress, hypoxia or oncogene activation, resulting in DNA repair, cell-cycle arrest, cell metabolism, autophagy, cell senescence as well as apoptosis.[9] Moreover, p53 is one of the most commonly mutated genes during the development of most human tumor types, highlighting its crucial role in carcinogenesis.[10] In the absence of p53 activity, cancer progress is accelerated, and resistance to chemotherapy is developed, leading to poor prognosis for patients.[11] It was previously shown that in response to death stimulus, p53 rapidly translocates into the mitochondria and triggers the first wave of cell death. This effect is followed by a slow wave of cell death because of the activation of transcription of p53-dependent apoptotic target genes.[12] Notably, the activity and subcellular distribution of p53 is regulated by many proteins. One of the most extensively studied regulators of p53 is the E3 ubiquitin protein ligase MDM2.[13]

Given that the changes in MDM2 levels influence the p53 signaling pathway, we postulated that there would be functional sequence variants in the promoter regions of the MDM2 gene. These variants would regulate p53-mediated apoptosis and may play an important role in individuals' risk of treatment-related toxicities. Recently, 2 common polymorphisms in the MDM2 promoter region, i.e., MDM2 c.14 + 309T > G (rs2279744, MDM2 SNP309) and MDM2 c.−461C > G (rs937282, MDM2 C1797G) were shown to affect its affinity for stimulatory protein 1 (SP1) and CAAT/enhancer binding protein α (C/EBP α), respectively, increasing the expression of MDM2.[14,15] In lung cancer, these genetic polymorphisms of MDM2 have been studied in relation to the risk of cancer and survival outcome but not in relation to toxicity.[16–21]

A common p53 polymorphism is located within the proline-rich domain of p53 at codon 72 (p53 p. Pro72Arg, rs1042522, p53 codon 72) and encodes either a proline residue (CCC) or an arginine residue (CGC). Dumont et al. observed that cells homozygous for arginine residue (Arg/Arg or G/G) display increased apoptotic potential and enhanced mitochondrial localization of p53.[22] They demonstrated that the enhanced mitochondrial localization of the Arg72 variant is associated with greater binding and ubiquitination of p53 by the MDM2 protein.[22] It is hypothesized that the Arg72 variant of p53 increases the risk of cell death because cells carrying this variant are more susceptible to platinum-based chemotherapy regimens. Conversely, the Arg72 variant may lead to increased toxicity due to the enhanced apoptotic potential of the Arg72 variant of p53 in normal bystander cells treated with platinum-based regimens. In this study, we investigated the association of the p53 p. Pro72Arg, MDM2 c.14 + 309T > G and MDM2 c.−461C > G polymorphisms with grade 3 or 4 toxicity in patients treated with platinum-based regimens for NSCLC.

Table 1. Characteristics of NSCLC patients

Patient characteristics	n (% of patients)
Total no. of patients	444
Median age, range (age at diagnosis, years)	57 (48)
Gender	
Male	318 (71.6)
Female	126 (28.4)
Performance status (PS) (n = 443)[a]	
0–1	425 (95.9)
2	18 (4.1)
Tumor-node-metastasis (TNM) stage	
IIIA	44 (9.9)
IIIB	131 (29.5)
IV	269 (60.6)
Histopathological type	
Adenocarcinoma	267 (60.1)
Squamous cell	89 (20.0)
Adenosquamocarcinoma	16 (3.6)
Other[b]	72 (16.2)
Chemotherapy regimens	
Platinum-navelbine	231 (52.0)
Platinum-gemcitabine	77 (17.3)
Platinum-paclitaxel	89 (20.0)
Platinum-docetaxel	22 (5.0)
Other platinum combinations	25 (5.6)
Smoking status	
Current	239 (53.8)
Former	9 (2.0)
Never	196 (44.1)
Response (n = 417)[a]	
Complete response (CR)	1 (0.2)
Partial response (PR)	75 (18.0)
Stable disease (SD)	290 (69.5)
Progressive disease (PD)	51 (12.2)
Toxicity outcomes	
Grade 3 or 4 toxicity (n = 382)[a]	142 (37.2)
Grade 3 or 4 hematologic toxicity (n = 401)[a]	129 (32.2)
Grade 3 or 4 gastrointestinal toxicity (n = 403)[a]	27 (6.7)
p53 p.Pro72Arg(rs1042522) (n = 441)[a]	
C/C	85 (19.3)
C/G	209 (47.4)
G/G	147 (33.3)
MDM2 c.−461C>G(rs937282) (n = 426)[a]	
C/C	226 (53.1)
C/G	157 (36.9)
G/G	43 (10.1)
MDM2 c.14 + 309T>G(rs2279744) (n = 430)[a]	
G/G	121 (28.1)
G/T	293 (68.1)
T/T	16 (3.7)

[a]available data from these indicated subjects.
[b]includes mixed cell, neuroendocrine carcinoma and unclassified carcinoma.

Results

Patient characteristics and outcomes

A total of 444 patients with advanced NSCLC enrolled in our study. Patient characteristics are shown in **Table 1**. Patients ranged from 32 to 80 y old, with a median of 57. 71.6% (318) were male. There were 239 smokers and 205 non-smokers. Data on performance status were available for 441 patients, 95.9% of whom showed a ZPS performance status of 1 or 0 (Zubrod-ECOG-WHO). All patients had advanced NSCLC (stage III or IV), with 9.9% at stage IIIA, 29.5% at stage IIIB and 60.6% at stage IV. The most common histology was adenocarcinoma (n = 267, 60.1%). Patients with squamous cell,

adenosquamocarcinoma or other histology represented 20% (89), 3.6% (16) and 16.2% (72) of the study population, respectively. All patients enrolled in the study had inoperable tumors and received platinum-based chemotherapy as first-line treatment. Fifty-two percent (231) of patients were treated with platinum-navelbine chemotherapy, 17.3% (77) with platinum-gemcitabine treatment, 20% (89) with platinum-paclitaxel treatment, and 5% (22) with platinum-docetaxel treatment. Only 5.6% (25) patients were treated with other platinum-based combinations. All chemotherapeutic drugs were administered intravenously.

Response data were available for 417 patients; 18.2% (76 cases) showed a response, with 0.2% (1) a complete response (CR) and 18.0% (75) a partial response (PR). The majority (81.8%, 341) of patients showed no response, with 69.5% (290) having stable disease (SD) and 12.2% (51) having progressive disease (PD).

The chemotherapy related toxicities were recorded, and incidences of grades 3 and 4 toxicities are shown in **Table 1**. Of the patients evaluated, 37.2% (142) suffered from grade 3 or 4 toxicity, 32.2% (129) had grade 3 or 4 hematologic toxicity, and 6.7% (27) experienced grade 3 or 4 gastrointestinal toxicity.

The p53 p. Pro72Arg, MDM2 c.14 + 309T > G and MDM2 c.−461C > G polymorphisms were identified in 441, 430 and 426 patients, respectively. In regard to the p53 p. Pro72Arg polymorphism, 19.3% (85) subjects were homozygous for the C allele (C/C genotype), 33.3% (147) were homozygous for the G allele (G/G genotype), and 47.4% (209) were heterozygous (C/G genotype). For the MDM2 c.14 + 309T > G polymorphism, 28.1% (121) subjects were homozygous for the G allele (G/G genotype), whereas 3.7% (16) were homozygous for the T allele (T/T genotype) and 68.1% (293) were heterozygous genotype (G/T). For the MDM2 c.−461C > G polymorphism, 53.1% (226) subjects were homozygous for the C allele (C/C genotype), 36.9% (157) were heterozygous (C/G genotype) and 10.1% (43) were homozygous for the G allele (G/G genotype).

No significant differences were observed in the overall survival rate for age, P = 0.989 (**Fig. S1A**); histological type, P = 0.423 (**Fig. S1B**); chemotherapy regimen, P = 0.528 (**Fig. S1D**) or patient response, P = 0.869 (**Fig. S1E**). However, patients at advanced stage IV had lower survival rates than those at lower stages (**Fig. S1C**).

Patient characteristics and clinical response rate

To investigate whether the objective response rate (ORR) and clinical benefit rate (CBR) were related to various patient characteristics, the patients were grouped according to median age (57 y), gender, PS, TNM stage, histology, smoking status, clinical toxicity outcome and genotypes of the 3 polymorphisms (p53 p. Pro72Arg, MDM2 c.14 + 309T > G and MDM2 c.−461C > G). The ORR and CBR were analyzed for the different groups (**Table 2**). The results showed that the patients above 57 y had a significantly higher ORR than the ≤ 57 y patients (P = 0.003) (**Table 2**). A higher ORR was observed in patients with squamous cell carcinoma compared to patients with adenocarcinoma (P < 0.001). We also found that there were no significant differences for CBRs in age, gender, PS class, TNM stage, histological type, smoking status, toxicity outcome and different genotypes (p53 p. Pro72Arg, MDM2 c.14 + 309T > G and MDM2 c.−461C > G) (**Table 2**). Some of the differences we found for patient characteristics using ORR do not hold when CBR is used. The difference between ORR and CBR in findings can be attributed to the large number in the SD group. Furthermore, neither ORR nor CBR was related to the toxicity rate or the 3 SNPs used in the study (**Table 2**). As a result, ORR or CBR will not be included in the analysis on the SNPs and toxicity outcome.

Association of polymorphisms with toxicity and survival outcome in NSCLC patients

We examined the association between the genotypes of three investigated SNPs and severe toxicities in the patients (**Table S1**). We observed that grade 3 or 4 gastrointestinal toxicity was significantly higher in patients who were heterozygous for the C allele of MDM2 c.−461C > G than G/G homozygotes (10.8% vs. 0; OR = 1.121, 95% CI = 1.062–1.184, P = 0.026) (**Table S1**).

After controlling for age, we found that younger patients (≤ 57 years) carrying the G/T genotype of the MDM2 c.14 + 309T > G polymorphism were more likely to experience hematologic toxicity (34.0% vs. 13.1%; adjusted OR = 3.578, 95% CI = 1.559–8.207; P = 0.003) and grade 3 or 4 overall toxicity (36.0% vs.18.0%; adjusted OR = 2.763, 95% CI = 1.293–5.905; P = 0.009) G/G homozygotes (**Table 3**). No statistically significant difference was found in elderly patients (**Table S2**).

For polymorphisms of the p53 p. Pro72Arg or MDM2 c.−461C > G polymorphisms, no significant associations were found between them and hematologic, gastrointestinal, or overall toxicity for younger patients (**Table 3**). In contrast, a significantly lower risk of grade 3 or 4 gastrointestinal toxicity was observed in elderly patients carrying at least a C allele of p53 p. Pro72Arg (G/C or C/C) (adjusted OR = 0.247, 95% CI = 0.076–0.810; P = 0.021) (**Table S2**). However, an association between MDM2 c.−461C > G associated and risk of grade 3 or 4 gastrointestinal toxicity was not found in these elderly patients (**Table S2**).

We also analyzed the association between the 3 SNPs and toxicity outcomes in patients with lung adenocarcinoma (**Table 4**). Interestingly, patients with the G/T variant of the MDM2 c.14 + 309T > G polymorphism showed increased rates of hematological toxicity (34.8% vs. 19.7%; adjusted OR = 2.247, 95% CI = 1.148–4.397; P = 0.018) and grade 3 or 4 overall toxicity (37.1% vs. 23.9%; adjusted OR = 1.957, 95% CI = 1.028–3.724; P = 0.041) than patients homozygous for the G/G variant (**Table 4**). Moreover, we found a statistically significant association between grade 3 or 4 gastrointestinal toxicity and the p53 p. Pro72Arg polymorphism (adjusted OR = 0.274, 95% CI = 0.101–0.746; P = 0.011) (**Table 4**). However, no significant association between the MDM2 c.−461C > G polymorphism and toxicity outcomes was observed in patients with adenocarcinoma (**Table 4**). After considering the Bonferroni correction for

Table 2. Association between patient characteristics and response to chemotherapy

Characteristics	n	CR, n	PR, n	SD, n	ORR, %	P-value	CBR, %	P-value
Age (years)								
≤ 57	208	0	26	153	12.5	0.003	86.1	0.287
> 57	209	1	49	137	23.9		89.5	
Gender								
Male	302	1	60	205	20.2	0.091	88.1	0.754
Female	115	0	15	85	13.0		87.0	
Performance status (PS)								
0–1	401	1	74	275	18.7	0.236	87.3	0.140
2	15	0	1	14	6.7		100	
TNM stage								
IIIA	42	1	11	27	28.6	0.163	92.9	0.530
IIIB	124	0	23	84	18.5		86.3	
IV	251	0	41	179	16.3		87.6	
Histologic type								
Adenocarcinoma	249	0	28	186	11.2	< 0.001	86.0	0.327
Squamous cell	85	1	26	51	31.8		91.8	
Smoking status								
Yes	236	0	49	156	20.7	0.125	86.7	0.639
No	181	1	26	133	14.9		88.4	
Toxicity outcome (hematologic + gastrointestinal toxicities)								
grade 0 to 2 toxicity	232	0	34	172	14.7	0.107	88.8	0.411
grade 3 or 4 toxicity	142	0	30	92	21.1		86.0	
p53 p.Pro72Arg (rs1042522)								
G/G	138	0	24	98	17.4	0.937	88.4	0.283
G/C	196	1	36	138	18.9		89.3	
C/C	80	0	15	51	18.8		82.5	
MDM2 c.14+309T>G(rs2279744)								
G/G	114	1	22	78	20.2	0.225	88.6	0.896
G/T	274	0	44	194	16.1		86.9	
T/T	16	0	5	9	31.3		87.5	
MDM2 c.-461C>G (rs937282)								
C/C	209	1	37	143	18.2	0.681	86.6	0.586
C/G	152	0	27	106	17.8		87.5	
G/G	40	0	5	32	12.5		92.5	

CR, Complete response; PR, partial response; SD, stable disease; ORR, objective response rate; CBR, clinical benefit rate.

multiple testing for each SNP, the association of MDM2 c. 14+ 309T > G with hematologic toxicity is still significant in the younger patient group.

Figure S2 presents the Kaplan-Meier curves of NSCLC patients according to their genetic polymorphisms. No associations were found between genetic polymorphisms (p53 p. Pro72Arg, MDM2 c.14 + 309T > G and MDM2 c.−461C > G) and overall survival rate for NSCLC patients (**Fig. S2**). The lack of associations between those genetic polymorphisms and progression-free survival in NSCLC patients suggests that these are not prognostic markers, but the associations of genetic polymorphisms with grade 3 or 4 toxicity outcomes suggests that they might act as possible biomarkers for adverse events following platinum-based chemotherapy treatment in advanced NSCLC patients.

Haplotype analysis

The pairwise linkage disequilibrium for the MDM2 c.−461C > G and MDM2 c.14 + 309T > G loci were calculated by the SHEsis software platform (D' = 0.655, r^2 = 0.287). The 3 common haplotypes, CG, GT and CT (MDM2 c.−461C > G–MDM2 c.14 + 309T > G, in order), were found to account for 93.7% of the patients. Because the CG haplotype has the highest haplotype frequency for MDM2 in the research population, determining the association between MDM2 polymorphisms and clinical toxicities is very complex. We set the CG haplotype of MDM2 as the reference group in this study. No significant difference in clinical toxicity was found among the MDM2 haplotype groups compared with the reference haplotype (**Table S3**). However, after controlling for age, we found that in the younger patients, the CT haplotype was associated with a higher incidence of hematologic toxicity (adjusted OR = 1.876, 95% CI = 1.033–3.406; P = 0.039) and grade 3 or 4 overall toxicity (adjusted OR = 2.109, 95% CI = 1.152–3.860; P = 0.016) compared with the most common haplotype, CG (haplotype frequency = 0.559) (**Table 5**).

A total of 8 diplotypes were found in the studied population. The 3 most common diplotypes were CG/CG, CG/CT, and CG/GT (MDM2 c.−461C > G–MDM2 c.14 + 309T >

Table 3. Association of the polymorphisms and grade 3 or 4 toxicity in young patients (age 57 and younger)

Genotype	n	Grade 3 or 4 toxicity n (%)	OR (95%CI)	P-value
Hematologic toxicity				
p53 p.Pro72Arg (rs1042522)				
G/G	73	20 (27.4)	1	
G/C	99	22 (22.2)	0.777 (0.380–1.589)	0.490
C/C	50	17 (34.0)	1.459 (0.652–3.267)	0.358
Allele of C (G/C+C/C)	149	39 (36.2)	0.974 (0.509–1.863)	0.937
MDM2 c.14+309T>G (rs2279744)				
G/G	61	8 (13.1)	1	
G/T	150	51 (34.0)	3.578 (1.559–8.207)	**0.003**
T/T	10	1 (10.0)	0.662 (0.073–5.967)	0.713
Allele of T (G/T+T/T)	160	52 (32.5)	3.295 (1.442–7.530)	**0.005**
MDM2 c.-461C>G (rs937282)				
G/G	21	5 (23.8)	1	
C/G	78	22 (28.2)	0.788 (0.250–2.483)	0.684
C/C	114	31 (27.2)	0.810 (0.266–2.465)	0.711
Allele of C (C/G+C/C)	192	53 (27.6)	0.801 (0.272–2.359)	0.687
Gastrointestinal toxicity				
p53 p.Pro72Arg (rs1042522)				
G/G	73	5 (6.8)	1	
C/G	99	8 (8.1)	1.207 (0.367–3.970)	0.757
C/C	50	1 (2.0)	0.300 (0.033–2.726)	0.285
Allele of C (G/C+C/C)	149	9 (6.0)	0.910 (0.285–2.911)	0.874
MDM2 c.14+309T>G (rs2279744)				
G/G	61	3 (4.9)	1	
G/T	150	10 (6.7)	1.618 (0.417–6.273)	0.486
T/T	10	1 (10.0)	2.425 (0.210–28.029)	0.478
Allele of T (G/T+T/T)	160	11 (6.9)	1.674 (0.438–6.395)	0.451
MDM2 c.-461C>G (rs937282)				
G/G	21	0 (0.0)	1	
C/G	78	7 (9.0)	1.099 (1.025–1.178)	0.340
C/C	114	7 (6.1)	1.065 (1.017–1.117)	0.595
Allele of C (C/G+C/C)	192	14	1.079 (1.037–1.122)	0.370
Overall toxicity				
p53 p.Pro72Arg (rs1042522)				
G/G	73	23 (31.5)	1	
G/C	99	25 (25.3)	0.775 (0.386–1.556)	0.474
C/C	50	18 (36.0)	1.382 (0.618–3.088)	0.431
Allele of C (G/C+C/C)	149	43 (28.9)	0.949 (0.503–1.791)	0.871
MDM2 c.14+309T>G (rs2279744)				
G/G	61	11 (18.0)	1	
G/T	150	54 (36.0)	2.763 (1.293–5.905)	**0.009**
T/T	10	2 (20.0)	1.213 (0.216–7.008)	0.815
Allele of T (G/T+T/T)	160	56 (35.0)	2.641 (1.243–5.614)	**0.012**
MDM2 c.-461C>G (rs937282)				
G/G	21	5 (23.8)	1	
C/G	78	24 (30.8)	0.759 (0.236–2.441)	0.644
C/C	114	36 (31.6)	0.677 (0.218–2.100)	0.500
Allele of C (C/G+C/C)	192	60 (31.3)	0.709 (0.236–2.134)	0.541

Note: Bold p-values are statistically significant.

G, in order). After controlling for age, CG/CT was associated with a incidence of hematologic toxicity (44.4% vs. 12.9%; adjusted OR = 5.665, 95% CI = 2.217–14.474; P < 0.001) and grade 3 or 4 overall toxicity (48.1% vs. 17.7%; adjusted OR = 4.949, 95% CI = 2.033–12.045; P < 0.001) than the CG/CG diplotype (**Table 6**). The association between the CG/CT diplotype of MDM2 and hematologic toxicity in younger patients was still significant after multiple testing adjustments. Additionally, we found that risk for grade 3 or 4 gastrointestinal toxicity was not associated with any diplotypes in younger patients (**Table 6**). Similar results were obtained from diplotype analysis regardless of age (**Table S4**; increased incidence of grade 3 or 4 hematologic toxicity, with adjusted ORs of 2.031, 95%CI = 1.129–3.654, P = 0.018; and increased incidence of grade 3 or 4 overall toxicity, with adjusted OR = 2.108, 95% CI = 1.171–3.796, P = 0.013). We note that the associations were enhanced in younger patients when age was considered (**Table 6**).

Table 4. Association of the polymorphisms with grade 3 or 4 toxicity in patients with adenocarcinoma

Genotype	n	Grade 3 or 4 toxicity n (%)	OR (95%CI)	P-value
Hematologic toxicity				
p53 p.Pro72Arg (rs1042522)				
G/G	87	28 (32.2)	1	
G/C	125	32 (25.6)	0.748 (0.403–1.388)	0.357
C/C	54	18 (33.3)	0.976 (0.468–2.035)	0.948
Allele of C (G/C+C/C)	179	50 (27.9)	0.819 (0.464–1.446)	0.491
MDM2 c.14+309T>G (rs2279744)				
G/G	71	14 (19.7)	1	
G/T	178	62 (34.8)	2.247 (1.148 -4.397)	**0.018**
T/T	12	3 (25.0)	1.392 (0.324–5.982)	0.656
Allele of T (G/T+T/T)	190	65 (34.2)	2.182 (1.120–4.253)	**0.022**
MDM2 c.-461C>G (rs937282)				
G/G	26	9 (34.6)	1	
C/G	95	26 (27.4)	1.305 (0.510–3.334)	0.579
C/C	136	42 (30.9)	1.136 (0.463–2.788)	0.781
Allele of C (C/G+C/C)	231	68	1.155 (0.482–2.768)	0.746
Gastrointestinal toxicity				
p53 p.Pro72Arg (rs1042522)				
G/G	87	11 (12.6)	1	
C/G	125	5 (4.0)	0.285 (0.093–0.870)	**0.028**
C/C	54	2 (3.7)	0.248 (0.052–1.184)	0.080
Allele of C (G/C+C/C)	179	7 (3.9)	0.274 (0.101–0.746)	**0.011**
MDM2 c.14+309T>G (rs2279744)				
G/G	71	5 (7.0)	1	
G/T	178	11 (6.2)	0.883 (0.292–0.271)	0.825
T/T	12	2 (16.7)	2.462 (0.403–15.024)	0.329
Allele of T (G/T+T/T)	190	13 (6.8)	0.978 (0.331–2.888)	0.968
MDM2 c.-461C>G (rs937282)				
G/G	26	0 (0.0)	1	
C/G	95	11 (11.6)	1.131 (1.052–1.216)	0.118
C/C	136	7 (5.1)	1.054 (1.014–1.096)	0.599
Allele of C (C/G+C/C)	231	18	1.085 (1.045–1.126)	0.231
Overall toxicity				
p53 p.Pro72Arg (rs1042522)				
G/G	87	33 (37.9)	1	
G/C	125	33 (26.4)	0.597 (0.323–1.104)	0.100
C/C	54	20 (37.0)	0.830 (0.402–1.713)	0.615
Allele of C (G/C+C/C)	179	53 (29.6)	0.670 (0.382–1.176)	0.163
MDM2 c.14+309T>G (rs2279744)				
G/G	71	17 (23.9)	1	
G/T	178	66 (37.1)	1.957 (1.028–3.724)	**0.041**
T/T	12	4 (33.3)	1.818 (0.452–7.308)	0.400
Allele of T (G/T+T/T)	190	70 (36.8)	1.947 (1.028–3.689)	**0.041**
MDM2 c.-461C>G (rs937282)				
G/G	26	9 (34.6)	1	
C/G	95	31 (32.6)	1.040 (0.406–2.662)	0.935
C/C	136	45 (33.3)	0.989 (0.398–2.453)	0.980
Allele of C (C/G+C/C)	231	76	0.976 (0.404–2.358)	0.956

Note: Bold p-values are statistically significant.

Discussion

Treatment selection based on biological markers is imperative for a personalized approach. The sequencing of the human genome has dramatically accelerated such research and allowed the identification of millions of single nucleotide polymorphisms (SNPs).[23] SNPs may play a crucial role in the expression level and activity of the corresponding gene products. When these polymorphisms occur in drug metabolism enzymes or transporters, the drug target or proteins involved in the repair of drug-induced lesions, the disposition of the drug may be altered and, consequently, its efficiency compromised or toxicity enhanced. For these reason, gene polymorphisms may lead to alterations in drug efficacy and treatment-related toxicities.

Despite the development of new therapeutic strategies, the prognosis of patients with advanced NSCLC remains poor. Platinum-based chemotherapy is still considered the standard first-line treatment for advanced NSCLC. SNPs have been suggested to be predictive markers of treatment response, toxicity and survival in lung cancer patients.[8,24,25] In this study, 3 investigated

Table 5. Association of the MDM2 haplotype with grade 3 or 4 toxicity in young patients (age 57 and younger)

Haplotype of MDM2 gene[a]	n	Grade 3 or 4 toxicity n (%)	OR (95%CI)	P-value
Hematologic toxicity				
CG	258	63 (24.4)	1	
GT	108	29 (26.9)	1.134 (0.673–1.911)	0.637
CT	63	24 (38.1)	1.876 (1.033–3.406)	**0.039**
GG	19	4 (21.1)	0.808 (0.253–2.582)	0.719
Gastrointestinal toxicity				
CG	258	16 (6.2)	1	
GT	108	7 (6.5)	1.064 (0.418–2.705)	0.897
CT	63	5 (7.9)	1.483 (0.510–4.310)	0.469
GG	19	0 (0)	—	—
Overall toxicity				
CG	258	72 (27.9)	1	
GT	108	31 (28.7)	1.051 (0.627–1.763)	0.850
CT	63	27 (42.9)	2.109 (1.152–3.860)	**0.016**
GG	19	4 (21.1)	0.665 (0.206–2.148)	0.495

Note: Bold p-values are statistically significant. [a]Haplotypes were composed of MDM2 c.−461C>G- c.14+309T>G, in order.

SNPs of p53 p. Pro72Arg, MDM2 c. 14+ 309T > G and MDM2 c.−461C > G were not associated with ORR and CBR in NSCLC patients during platinum-based chemotherapy. We found a statistically significant difference in clinical response to chemotherapy between subgroups of ≤ 57 and above 57 y (**Table 2**). There was different with the previous studies. We speculated that the possible reason may be the application of different borderline, but it should be further investigated. Meanwhile, higher ORR was observed in patients with squamous cell carcinoma compared to patients with non-squamous cell carcinoma (**Table 2**). The result may be due to chemotherapy regimens of advanced NSCLC patients without Pemetrexed and EGFR inhibitor. The Pemetrexed and EGFR inhibitor do provide a better clinical efficacy for advanced NSCLC patients with adenocarcinoma histological type, but they do not improve the response rate for patients with squamous histological type.[26]

Table 6. Association of the MDM2 diplotype with grade 3 or 4 toxicity in young patients (age 57 and younger)

Diplotype of MDM2 gene[a]	n	Grade 3 or 4 toxicity n (%)	OR (95%CI)	P-value
Hematologic toxicity				
CG/CG	62	8 (12.9)	1	
CG/CT	54	24 (44.4)	5.665 (2.217–14.474)	< **0.001**
CG/GG	1	0 (0)	—	—
CG/GT	79	23 (29.1)	2.926 (1.184–7.231)	**0.020**
CT/CT	2	0 (0)	—	—
CT/GT	5	0 (0)	—	—
GG/GT	18	4 (22.2)	1.935 (0.496–7.549)	0.342
GT/GT	3	1 (33.3)	2.715 (0.217–33.941)	0.438
Gastrointestinal toxicity				
CG/CG	62	3 (4.8)	1	
CG/CT	54	4 (7.4)	1.969 (0.404–9.598)	0.402
CG/GG	1	0 (0)	—	—
CG/GT	79	6 (7.6)	1.925 (0.445–8.322)	0.380
CT/CT	2	0 (0)	-	—
CT/GT	5	1 (20.0)	3.971 (0.301–52.330)	0.295
GG/GT	18	0 (0)	—	—
GT/GT	3	0 (0)	—	—
Overall toxicity				
CG/CG	62	11 (17.7)	1	
CG/CT	54	26 (48.1)	4.949 (2.033–12.045)	< **0.001**
CG/GG	1	0 (0)	—	
CG/GT	79	24 (30.4)	2.139 (0.925–4.951)	0.076
CT/CT	2	0 (0)	—	—
CT/GT	5	1 (20.0)	0.886 (0.088–8.882)	0.918
GG/GT	18	4 (20.2)	1.344 (0.355–5.089)	0.663
GT/GT	3	1 (33.3)	3.497 (0.196–62.418)	0.395

Note: Bold p-values are statistically significant. [a]Diplotypes were composed of MDM2 c.−461C>G–c.14+309T>G, in order.

Moreover, our result is consistent with a recent study which shows that response rate after platinum-based chemotherapy was significantly higher in advanced NSCLC patients with squamous carcinoma than with adenocarcinoma.[27]

Platinum-related toxicity, including hematologic and gastrointestinal toxicities, remains challenges in this treatment. A considerable number of studies have focused on the association between chemotherapy-related toxicity and polymorphisms in DNA repair genes.[8,24,25] Here, we focused on the association between incidence of toxicities in patients treated with platinum-based combination chemotherapy and polymorphisms of the p53 and MDM2 genes, which are involved in p53-mediated apoptosis in normal bystander cells suffering from platinum-based regimens.

In this study, we investigated whether the SNPs of p53 p. Pro72Arg, MDM2 c. 14+ 309T > G and MDM2 c.−461C > G were associated with the toxicity of platinum-based chemotherapy in advanced NSCLC patients. We found that MDM2 14 + C 309T > G was associated with toxicity in advanced NSCLC patients receiving platinum chemotherapy, especially young patients or for patients with adenocarcinoma (**Tables 3** and **4**). Furthermore, we observed that the incidence of gastrointestinal toxicity in patients heterozygous for the MDM2 c.−461C > G polymorphism was also significantly higher than in those with the G/G genotype. In addition, a statistically significant association was found between the occurrence of severe platinum chemotherapy-related toxicities in both the haplotype and diplotype analysis (**Tables 5** and **6**).

MDM2 c.14 + 309T > G and MDM2 c.−461C > G are well-studied functional SNPs in the promoter region of the MDM2 gene. MDM2 c.14 + 309T > G is located in the region of a p53-dependent promoter (P2 promoter), and MDM2 c.−461C > G is located upstream of a p53-independent constitutive P1 promoter.[13] Numerous epidemiological studies have focused on the association between MDM2 c.14 + 309T > G and MDM2 c.−461C > G SNPs and susceptibility to and survival of lung cancer, but the conclusions were not consistent.[16–21,28,29] In the present study, we observed that the heterozygous MDM2 c.14 + 309T > G genotype was associated with increased risk of severe hematologic and overall toxicities in NSCLC patients treated with platinum-based regimens. However, a higher rate of gastrointestinal toxicity was observed in patients heterozygous for the C allele of MDM2 c.−461C > G than those homozygous for the G allele. In vitro studies have demonstrated that the G allele of MDM2 c.14 + 309T > G could increase the steady-state expression of mRNA through enhancing the affinity of the Sp1 transcription activator for a putative binding site and thereby reducing basal p53 levels.[15,30] Wang et al. reported that the G allele of MDM2 c. −461C > G has a high promoter activity for MDM2 mRNA transcription based on in vitro luciferase assays in various cell lines.[14] Therefore, the distinct associations of toxicity outcomes with MDM2 c.14 + 309T > G and MDM2 c.−461C > G may be due to the specific types and areas of normal bystander cells and their regulation of MDM2 expression. Basal levels of MDM2 protein could be increased to a certain extent in normal bystander cells with the MDM2 c.14 + 309T > G G/T genotype, which in turn attenuates the p53 response to a variety of DNA damage induced by platinum-based chemotherapy regimens. Lalonde et al. reported that the constitutive expression levels of MDM2 might be regulated by multiple functional promoter SNPs that subsequently suppress the regulation of MDM2 c−461C > G.[13] We used a haplotype and diplotype-based approach to evaluate their association with the occurrence of severe hematologic and overall toxicities. We found a statistically significant association between the CG/CT (MDM2 c−461C > G-MDM2 c.14 + 309T > G, in order) and increased incidence of severe hematologic and overall toxicities. The results indicate that the combination of 2 functional SNPs could increase the risk of severe hematologic and overall toxicity.

A common p53 polymorphism, p53 encodes either a proline residue (CCC) or an arginine residue (CGC) at the 72nd residue (p.Pro72Arg). Increased apoptotic potential response to genotoxic agents in cells homozygous for the G allele (Arg allele) has been described previously.[22] Analysis of severe toxicity revealed that patients with the C allele were associated with lower incidence of gastrointestinal toxicity than those with the G/G genotype. This suggests that the Arg72 variant (the G variant) may predict for severe toxicities in normal bystander cells treated with platinum-based regimens. However, no association between p53 p.Pro72Arg and the occurrence of severe hematologic and overall toxicity was observed in the present study.

Earlier studies showed that p53 p. Pro72Arg and MDM2 c.14 + 309T > G SNPs are associated with severe neutropenia in patients with breast and ovarian cancer during cisplatin-based chemotherapy.[31,32] Recently, Wang et al observed associations between p53 p. Pro72Arg and MDM2 c.14 + 309T > G and grade 3 or 4 neutropenia in extensive-stage small-cell lung cancer (SCLC) patients treated with etoposide and cisplatin.[3] They reported that p53 p. Pro72Arg G/G and MDM2 c.14 + 309T > G G/G genotypes were associated with the lowest incidence of grade 3 or 4 neutropenia in SCLC patients.[33] In current study, we found that the MDM2 c.14 + 309T > G polymorphism is associated with grade 3 or 4 hematologic toxicity in advanced NSCLC patients treated with platinum-based chemotherapy. This result is consistent with previous reports. In contrast, patients with the p53 p.Pro72Arg G/G genotype were associated with higher incidence of gastrointestinal toxicity than those with the C allele. In addition, we found that the incidence of gastrointestinal toxicity in patients heterozygous for the MDM2 c.−461C > G polymorphism was also significantly higher than in those with the G/G genotype. Because of the limited number of gastrointestinal toxicity events, a larger sample is needed to validate this result.

In conclusion, our results suggested that the MDM2 c.14 + 309T > G polymorphism is associated with grade 3 or 4 hematologic and overall toxicity in advanced NSCLC patients treated with platinum-based chemotherapy. The p53 p.Pro72Arg and MDM2 c.−461C > G were associated with the risk of severe gastrointestinal toxicity in this study. If confirmed by independent studies, these findings may have potential clinical utility in assessing the toxicity risk and individualizing therapy.

Materials and Methods

Patients and data collection

All patients with newly diagnosed advanced NSCLC were enrolled for the study at The Second Hospital, Nanjing Medical University and Jiangsu Cancer Hospital-Jiangsu Institute of Cancer Research between August 2009 and September 2012. All subjects were unrelated ethnic Han Chinese. Patients receiving radiation therapy were excluded in this study. All subjects were histologically confirmed as having stage IIIA–IV tumors that were inoperable but received first-line platinum-based chemotherapy including cisplatin and carboplatin. They were given (1) 25 mg/m^2 of navelbine on day 1 and day 8 every 3 weeks in combination with 75 mg/m^2 cisplatin or carboplatin AUC 5, both administered on day 1 every 3 weeks; (2) 1,250 mg/m^2 gemcitabine on days 1 and 8 every 3 weeks in combination with 75 mg/m^2 cisplatin or carboplatin AUC 5, both administered on day 1, every 3 weeks; (3) 175 mg/m^2 Taxol on day 1 every 3 weeks in combination with 75 mg/m^2 cisplatin or carboplatin AUC 5, both administered on day 1 every 3 weeks; (4) 75 mg/m^2 docetaxel on day 1 every 3 weeks in combination with 75 mg/m^2 cisplatin on day 1 every 3 weeks. All drugs were administered intravenously, and patients were treated for 2 to 6 cycles. Patients had to be available for follow-up and informed consent was provided. Clinical data were systematically recorded at the time of admission, including age when diagnosed, sex, smoking history, family history, clinical stage, and tumor histology.

Responses to treatment were evaluated by solid tumor guidelines (RECIST), which classify the responses into the following 4 categories: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). ORR and CBR were analyzed in the present study. ORR is the percentage of patients who experienced CR and PR after treatment, and CBR is the percentage of patients with CR, PR and SD.

Toxicity incidences during first-line chemotherapy were assessed by the National Cancer Institute's Common Toxicity Criteria version 3.0 (http://ctep.cancer.gov) and were recorded after first 2 cycles of chemotherapy by an investigator who was blind to the status of the patients. The toxicities included leukocytopenia, anemia, thrombocytopenia, nausea, and vomiting. Toxicity outcomes were grouped into any grade 3 or 4 toxicity, any grade 3 or 4 hematologic toxicity, and any grade 3 or 4 gastrointestinal toxicity. The toxicity outcome in each of these groups was dichotomized by the presence or absence of grade 3 or 4 toxicity during the first-line treatment. The study protocol was approved by the ethics committees of The Second Clinical School and The Second Hospital of Nanjing Medical University.

Genotyping

Peripheral blood samples were obtained from all recruited NSCLC patients after signing informed consent forms. Genomic DNA was extracted from peripheral blood samples using the QIAamp DNA Blood Kit (Qiagen). The p53 p. Pro72Arg, MDM2 c.14 + 309T > G and MDM2 c.−461C > G genotypes were determined by PCR-RFLP methods as described previously,[34] and followed by the direct sequencing of 10% samples with the heterozygous or homozygous variant. Direct sequencing of the PCR product was performed with BigDye Terminator v3.1 kit (The ABI PRISM 3100 Genetic Analyzer, Applied Biosystems).

Statistical Analysis

The Hardy-Weinberg equilibrium of each polymorphism distribution was tested by a goodness-of-fit χ^2 test. For the pair wise linkage disequilibrium analysis, D' and r^2 for the observed polymorphisms in MDM2 gene were calculated by the SHEsis software platform (http://analysis2.bio-x.cn/myAnalysis.php).[35] Individual haplotype frequencies were estimated from the observed genotype data using the PHASE 2.1.1 program (version 2.1.1) involving the implementation of a Bayesian approach for reconstructing haplotypes from population genotype data.[36]

Comparison of categorical variables of patient characteristics was performed by χ^2 test or Fisher's exact test. The associations between each genetic polymorphism/haplotype/diplotype and toxicity outcomes (grade 3 or 4 toxicity) were estimated by odds ratios (OR) and their 95% confidence intervals (95% CI), which were calculated using unconditional logistic regression. The covariates of treatment responses included sex, age at diagnosis, performance stage (PS), type of chemotherapy regimen, tumor/node/metastasis (TNM) stage and histological type, and the covariates of toxicity outcomes included sex, PS and type of chemotherapy regimen. For analysis of overall survival, Kaplan-Meier curves were constructed. The estimates for different subgroups were calculated by the log-rank method. P values were 2-sided, and a level of 0.05 was considered statistically significant. When multiple comparisons of the data were performed, the Bonferroni correction was applied. All statistical analyses were carried out by Stata version 11 (StataCorp LP, College Station, TX).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

This study was supported by the 07-B-023 and H200824 grants from the Health Department of Jiangsu province to DT.

Supplemental Material

Supplemental data for this article can be accessed on the publisher's website.

References

1. Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin 2011; 61:212-36; PMID:21685461; http://dx.doi.org/10.3322/caac.20121
2. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, Zhu J, Johnson DH, Eastern Cooperative Oncology G. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 2002; 346:92-8; PMID:11784875; http://dx.doi.org/10.1056/NEJMoa011954
3. Scagliotti GV, Parikh P, von Pawel J, Biesma B, Vansteenkiste J, Manegold C, Serwatowski P, Gatzemeier U, Digumarti R, Zukin M, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. J Clin Oncol: Official J Am Soc Clin Oncol 2008; 26:3543-51; PMID:18506025; http://dx.doi.org/10.1200/JCO.2007.15.0375
4. Zamble DB, Lippard SJ. Cisplatin and DNA repair in cancer chemotherapy. Trends Biochem Sci 1995; 20:435-9; PMID:8533159; http://dx.doi.org/10.1016/S0968-0004(00)89095-7
5. Innocenti F, Undevia SD, Iyer L, Chen PX, Das S, Kocherginsky M, Karrison T, Janisch L, Ramírez J, Rudin CM. Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. J Clin Oncol 2004; 22:1382-8; PMID:15007088; http://dx.doi.org/10.1200/JCO.2004.07.173
6. Han B, Gao G, Wu W, Gao Z, Zhao X, Li L, Qiao R, Chen H, Wei Q, Wu J, et al. Association of ABCC2 polymorphisms with platinum-based chemotherapy response and severe toxicity in non-small cell lung cancer patients. Lung Cancer 2011; 72:238-43; PMID:20943283; http://dx.doi.org/10.1016/j.lungcan.2010.09.001
7. Pan J-H, Han J-X, Wu J-M, Huang H-N, Yu Q-Z, Sheng L-J. MDR1 single nucleotide polymorphism G2677T/A and haplotype are correlated with response to docetaxel-cisplatin chemotherapy in patients with non-small-cell lung cancer. Respiration 2008; 78:49-55; PMID:18812689; http://dx.doi.org/10.1159/000158454
8. Zhang L, Gao G, Li X, Ren S, Li A, Xu J, Zhang J, Zhou C. Association between single nucleotide polymorphisms (SNPs) and toxicity of advanced non-small-cell lung cancer patients treated with chemotherapy. PloS One 2012; 7:e48350; PMID:23118991; http://dx.doi.org/10.1371/journal.pone.0048350
9. Vousden KH, Lane DP. p53 in health and disease. Nat Rev Mol Cell Biol 2007; 8:275-83; PMID:17380161; http://dx.doi.org/10.1038/nrm2147
10. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. Science 1991; 253:49-53; PMID:1905840; http://dx.doi.org/10.1126/science.1905840
11. Skaug V, Ryberg D, Arab EHKMO, Stangeland L, Myking AO, Haugen A. p53 mutations in defined structural and functional domains are related to poor clinical outcome in non-small cell lung cancer patients. Clin Cancer Res 2000; 6:1031-7; PMID:10741731
12. Erster S, Mihara M, Kim RH, Petrenko O, Moll UM. In vivo mitochondrial p53 translocation triggers a rapid first wave of cell death in response to DNA damage that can precede p53 target gene activation. Mol Cell Biol 2004; 24:6728-41; PMID:15254240; http://dx.doi.org/10.1128/MCB.24.15.6728-6741.2004
13. Lalonde ME, Ouimet M, Lariviere M, Kritikou EA, Sinnett D. Identification of functional DNA variants in the constitutive promoter region of MDM2. Hum Genomics 2012; 6:15; PMID:23244604; http://dx.doi.org/10.1186/1479-7364-6-15
14. Wang M, Zhang Z, Zhu H, Fu G, Wang S, Wu D, Zhou J, Wei Q, Zhang Z. A novel functional polymorphism C1797G in the MDM2 promoter is associated with risk of bladder cancer in a Chinese population. Clin Cancer Res: Official J Am Assoc Cancer Res 2008; 14:3633-40; PMID:18519798; http://dx.doi.org/10.1158/1078-0432.CCR-07-5155
15. Bond GL, Hu W, Bond EE, Robins H, Lutzker SG, Arva NC, Bargonetti J, Bartel F, Taubert H, Wuerl P, et al. A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. Cell 2004; 119:591-602; PMID:15550242; http://dx.doi.org/10.1016/j.cell.2004.11.022
16. Dong J, Ren B, Hu Z, Chen J, Hu L, Dai J, Jin G, Xu L, Shen H. MDM2 SNP309 contributes to non-small cell lung cancer survival in Chinese. Mol Carcinogenesis 2011; 50:433-8; PMID:21268124; http://dx.doi.org/10.1002/mc.20727
17. Chua HW, Ng D, Choo S, Lum SS, Li H, Soh LY, Sabapathy K, Seow A. Effect of MDM2 SNP309 and p53 codon 72 polymorphisms on lung cancer risk and survival among non-smoking Chinese women in Singapore. BMC Cancer 2010; 10:88; PMID:20219101; http://dx.doi.org/10.1186/1471-2407-10-88
18. Han JY, Lee GK, Jang DH, Lee SY, Lee JS. Association of p53 codon 72 polymorphism and MDM2 SNP309 with clinical outcome of advanced nonsmall cell lung cancer. Cancer 2008; 113:799-807; PMID:18618574; http://dx.doi.org/10.1002/cncr.23668
19. Wilkening S, Bermejo JL, Hemminki K. MDM2 SNP309 and cancer risk: a combined analysis. Carcinogenesis 2007; 28:2262-7; PMID:17827408; http://dx.doi.org/10.1093/carcin/bgm191
20. Zhang X, Miao X, Guo Y, Tan W, Zhou Y, Sun T, Wang Y, Lin D. Genetic polymorphisms in cell cycle regulatory genes MDM2 and TP53 are associated with susceptibility to lung cancer. HumMutat 2006; 27:110-7; PMID:16287156; http://dx.doi.org/10.1002/humu.20277
21. Lind H, Zienolddiny S, Ekstrom PO, Skaug V, Haugen A. Association of a functional polymorphism in the promoter of the MDM2 gene with risk of non-small cell lung cancer. Int J Cancer J Int Cancer 2006; 119:718-21; PMID:16496380; http://dx.doi.org/10.1002/ijc.21872
22. Dumont P, Leu JI, Della Pietra AC 3rd, George DL, Murphy M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat Genetics 2003; 33:357-65; PMID:12567188; http://dx.doi.org/10.1038/ng1093
23. Genomes Project C, Abecasis GR, Altshuler D, Auton A, Brooks LD, Du-rbin RM, Gibbs RA, Hurles ME, McVean GA. A map of human genome variation from population-scale sequencing. Nature 2010; 467:1061-73; PMID:20981092; http://dx.doi.org/10.1038/nature09534
24. Nancy P, Sakkaraiappan R, Vinay KJ. Impact of genetic polymorphisms in DNA repair enzymes on drug resistance in lung cancer. Clin Lung Cancer 2004; 6:79-82; PMID:15476592; http://dx.doi.org/10.1016/S1525-7304(11)70205-4
25. Chen S, Huo X, Lin Y, Ban H, Lin Y, Li W, Zhang B, Au WW, Xu X. Association of MDR1 and ERCC1 polymorphisms with response and toxicity to cisplatin-based chemotherapy in non-small-cell lung cancer patients. Int J Hyg Environ Health 2010; 213:140-5; PMID:20189873; http://dx.doi.org/10.1016/j.ijheh.2010.01.004
26 Ciuleanu T, Brodowicz T, Zielinski C, Kim JH, Krzakowski M, Laack E, Wu YL, Bover I, Begbie S, Tzekova V, et al. Maintenance pemetrexed plus best supportive care versus placebo plus best supportive care for non-small-cell lung cancer: a randomised, double-blind, phase 3 study. Lancet 2009; 374 (9699):1432-40; PMID:19767093; http://dx.doi.org/10.1016/S0140-6736(09)61497-5
27. Li CH, Liu MY, Liu W, Li DD, Cai L. Randomized control study of nedaplatin or cisplatin concomitant with other chemotherapy in the treatment of advanced non-small cell lung cancer. Asian Pac J Cancer Prev 2014; 15 (2): 731-6; PMID:24568487; http://dx.doi.org/10.7314/APJCP.2014.15.2.731
28. Li G, Zhai X, Zhang Z, Chamberlain RM, Spitz MR, Wei Q. MDM2 gene promoter polymorphisms and risk of lung cancer: a case-control analysis. Carcinogenesis 2006; 27:2028-33; PMID:16675470; http://dx.doi.org/10.1093/carcin/bgl047
29. Hu Z, Ma H, Lu D, Qian J, Zhou J, Chen Y, Xu L, Wang X, Wei Q, Shen H. Genetic variants in the MDM2 promoter and lung cancer risk in a Chinese population. Int J Cancer J Int Cancer 2006; 118:1275-8; PMID:16152608; http://dx.doi.org/10.1002/ijc.21463
30. Arva NC, Gopen TR, Talbott KE, Campbell LE, Chicas A, White DE, Bond GL, Levine AJ, Bargonetti J. A chromatin-associated and transcriptionally inactive p53-Mdm2 complex occurs in mdm2 SNP309 homozygous cells. J Biol Chem 2005; 280:26776-87; PMID:15908423; http://dx.doi.org/10.1074/jbc.M505203200
31. Khrunin AV, Moisseev A, Gorbunova V, Limborska S. Genetic polymorphisms and the efficacy and toxicity of cisplatin-based chemotherapy in ovarian cancer patients. Pharmacogenomics 2010; 10: 54-61; PMID:19786980
32. Okishiro M, Kim SJ, Tsunashima R, Nakayama T, Shimazu K, Shimomura A, Maruyama N, Tamaki Y, Noguchi S. MDM2 SNP309 and TP53 R72P associated with severe and febrile neutropenia in breast cancer patients treated with 5-FU/epirubicin/cyclophosphamide. Breast Cancer Res Treat 2012; 132: 947-53; PMID:21706156; http://dx.doi.org/10.1007/s10549-011-1637-5
33. Wang X, Wang YZ, Ma KW, Chen X, Li W. MDM2 rs2279744 and TP53 rs1042522 Polymorphisms Associated with Etoposide- and cisplatin-induced grade III/IV neutropenia in Chinese extensive-stage small-cell lung cancer patients. Oncol Res Treat 2014; 37(4): 176-80; PMID:24732641; http://dx.doi.org/10.1159/000360785
34. Xu B, Mi Y-Y, Min Z-C, Cheng G, Tong N, Tao J, Li P-C, Wang M-L, Tang J-L, Zhang Z-D. p53 codon 72 increased biochemical recurrence risk after radical prostatectomy in a southern Chinese population. Urol Ints 2010; 85:401-5; PMID:20664183; http://dx.doi.org/10.1159/000315991
35. Yong Y, Lin H. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 2005; 15:97-8; PMID:15740637; http://dx.doi.org/10.1038/sj.cr.7290272
36. Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 2003; 73:1162-9; PMID:14574645; http://dx.doi.org/10.1086/379378