Original Article

Association between well-characterized lung cancer lncRNA polymorphisms and platinum-based chemotherapy toxicity in Chinese patients with lung cancer

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Abstract

Platinum-based chemotherapy is the standard first-line treatment for most lung cancer patients. However, the toxicity induced by platinum-based chemotherapy greatly impedes its clinical use. Previous studies showed that long non-coding RNAs (lncRNAs) with over 200 nucleotides in length affect drug response and toxicity. In the present study, we investigated the association of well-characterized lung cancer lncRNA polymorphisms with platinum-based chemotherapy toxicity in Chinese patients with lung cancer. A total of 467 lung cancer patients treated with platinum-based chemotherapy for at least two cycles were recruited. We primarily focused on gastrointestinal and hematological toxicities. A total of 14 potentially functional polymorphisms within 8 lncRNAs (*HOTTIP*, *HOTAIT*, *H19*, *ANRIL*, *CCAT2*, *MALAT1*, *MEG3*, and *POLR2E*) were genotyped. Unconditional logistical regression analysis was conducted to assess the associations. Gene-gene and gene-environment interactions were identified using the software generalized multifactor dimensionality reduction (GMDR). *ANRIL* rs1333049 was associated with severe overall toxicity in an additive model (adjusted OR=0.723, 95% CI=0.541–0.965, *P*=0.028). *ANRIL* rs1333049 was also associated with severe gastrointestinal toxicity in both the additive (adjusted OR=0.690, 95% CI=0.489–0.974, *P*=0.035) and dominant (adjusted OR=0.558, 95% CI=0.335–0.931, *P*=0.025) models. *MEG3* rs116907618 was associated with severe gastrointestinal toxicity in an additive model (adjusted OR=1.717, 95% CI=1.007–2.927, *P*=0.047). GMDR identified the three-factor interaction model of *POLR2E* rs3787016-*HOTTIP* rs3807598-chemotherapy regimen as the best predictive model for hematological toxicity. In conclusion, *ANRIL* and *MEG3* genetic polymorphisms are associated with severe platinum toxicity and could be considered as biomarkers for pretreatment evaluation in Chinese patients with lung cancer.

Keywords: lung cancer; platinum-based chemotherapy; gastrointestinal toxicity; hematological toxicity; long non-coding RNA (lncRNA); polymorphism; Chinese patients

Acta Pharmacologica Sinica (2017) 38: 581–590; doi: 10.1038/aps.2016.164; published online 6 Mar 2017

Introduction

Lung cancer is one of the most commonly diagnosed cancers and is the leading cause of cancer death worldwide^[1]. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of lung cancer cases. When diagnosed with lung cancer, most patients are typically in the late stages. Although sig-

nificant progress in diagnosis and treatment has been made, the 5-year overall survival rate of lung cancer patients is only approximately 18%^[2].

Platinum-based chemotherapy is the standard care for NSCLC patients, particularly those who are in advanced stages. However, unpredictable severe side effects, including gastrointestinal and hematologic toxicities, significantly impede the effective clinical use of platinum-based chemotherapy. The gastrointestinal toxicity, particularly severe nausea and vomiting, often results in dehydration, inappetence,

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Received 2016-08-11 Accepted 2016-11-23

malnutrition, and a reduction in treatment compliance. The main clinical symptoms of hematological toxicity include hypochromia, leukopenia, neutropenia and thrombocytopenia. According to a survey of advanced NSCLC patients, to whom a choice between supportive care and chemotherapy was presented, many would not choose chemotherapy for its likely survival benefit of 3 months but would if this treatment improved their quality of life^[3]. The incidence and severity of toxicities vary greatly between individuals^[4], which suggests the need to obtain predictive markers that can identify potential chemotherapy beneficiaries with minimal toxicity.

Long non-coding RNAs (lncRNAs) are non-protein coding transcripts of over 200 nucleotides in length. Although the mechanisms of action for most of these molecules remain unknown, increasing evidence indicates that lncRNAs are important regulators in diverse biological processes, including cell growth and apoptosis. Previous studies also indicated that lncRNA expression affected drug response and toxicity^[5, 6]. lncRNA NR_045623 and NR_028291 have been implicated in benzene hematoxicity^[7]. In addition, single nucleotide polymorphisms (SNPs) can affect gene expression and function, partly accounting for individual differences in drug toxicity^[8]. Notably, more than one-third of trait/disease-associated variants identified by genome-wide association studies were mapped to non-coding intervals^[9]. Those SNPs, particularly those in lncRNAs, may affect drug toxicity.

HOTTIP, *HOTAIT*, *H19*, *ANRIL*, *CCAT2*, *MALAT1*, *MEG3*, and *POLR2E* are the most studied lncRNAs involved in lung cancer tumorigenesis and drug response. In previous studies, we observed that several SNPs in those lncRNAs were associated with lung cancer susceptibility and platinum-based chemotherapy response^[10]. In the present study, we evaluated the association of those SNPs with platinum-based chemotherapy toxicity in lung cancer patients.

Materials and methods

Study subjects

A total of 467 lung cancer patients were enrolled in the study. All subjects were at least 18 years old and were genetically unrelated. These patients were recruited from Xiangya Hospital of Central South University (Changsha, China) or the Affiliated Cancer Hospital of Central South University (Changsha, China) between November 2011 and May 2013.

All patients were histologically or cytologically diagnosed with primary lung cancer. The details of criteria for patient recruitment and chemotherapy regimens were described elsewhere^[10]. The patient charts were reviewed to extract data on the experienced toxicities. The severity of toxicity was assessed according to the National Cancer Institute Common Toxicity Criteria version 3.0^[11]. The investigators were blinded to the polymorphism status of the patients. We primarily focused on nausea, vomiting, hypochromia, leukopenia, neutropenia and thrombocytopenia. Severe gastrointestinal toxicity was grade 3 or 4 nausea and vomiting. Severe hematological toxicity included grade 3 or 4 hypochromia, leukopenia, neutropenia and thrombocytopenia. Patients who experienced

any type of the grade 3 or 4 toxicities described above were defined as suffering severe overall toxicity. The study protocol was approved by the Ethics Committee of Xiangya School of Medicine, Central South University (registration CTXY-1100082 and CTXY-110008-3). We also registered in the Chinese Clinical Trial Registry (registration ChiCTR-RO-12002873 and ChiCTR-RCC-12002830).

DNA extraction, SNP selection and genotyping

Genomic DNA was extracted from 5 mL venous blood using the FlexiGene DNA Kit (Qiagen, Hilden, Germany) or the Genomic DNA Purification Kit (Promega, Madison, WI, USA) following standard protocols. The DNA samples were stored at -20 °C until further use. According to the data from the HapMap database, 1000 Genomes database and previous studies, a total of 14 potentially functional SNPs or tag SNPs with minor allele frequency (MAF) >0.05 in Chinese population in 8 lncRNAs [rs1859168, rs5883064, and rs3807598 in *HOXA* distal transcript antisense RNA (*HOTTIP*); rs4759314, rs7958904 and rs1899663 in *HOX* transcript antisense intergenic RNA (*HOTAIR*); rs2839698 and rs2107425 in *H19*; rs10120688 and rs1333049 in *CDKN2B* antisense RNA 1 (*ANRIL*); rs6983267 in colon cancer-associated transcript 2 (*CCAT2*); rs619586 in metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*); rs116907618 in maternally expressed gene 3 (*MEG3*); and rs3787016 in polymerase (RNA) II subunit E (*POLR2E*)] were selected^[10]. These polymorphisms were genotyped using the Sequenom MassARRAY System (Sequenom, San Diego, CA, USA).

Statistical analysis

All statistical analyses were performed using PASW Statistics v18.0 software (IBM Co, Armonk, NY, USA), generalized multifactor dimensionality reduction (GMDR v0.9), and PLINK 1.9 (<http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml>). All tests were two-sided, and the criterion of statistical significance was set at $P < 0.05$. The chi-square test was used to assess differences in proportions between groups for the categorical variables. The Hardy-Weinberg equilibrium was calculated using the chi-square test. Unconditional logistical regression analysis was conducted to calculate the odds ratio (OR) and 95% confidence interval (95% CI) with adjustments for age, sex, smoking status, stage, performance score (PS), platinum dose, chemotherapy interval, preventive treatment, the time of examining the blood for hematotoxicity, histological type and chemotherapy regimen. Gene-gene and gene-environment interactions were identified using GMDR^[12]. A ten-fold cross-validation was set. Confounding factors, including age, sex, smoking status, stage, PS, platinum dose, chemotherapy interval, preventive treatment, the time of examining the blood for hematotoxicity, histological type and chemotherapy regimen, were included as covariates for gene-gene interaction analysis.

Results

Patient characteristics and toxicity outcomes

All 467 subjects were cytologically or histologically confirmed

Table 1. Clinical characteristics of lung cancer patients.

Characteristics	Stratification	n (%)
Total number of patients		467 (100)
Median age (range)	57 (20–80)	
	<57	234 (50.1)
	≥57	233 (49.9)
Sex	Male	371 (79.4)
	Female	96 (20.6)
History of smoking	Yes	286 (61.2)
	No	181 (38.8)
Histological type	NSCLC	371 (79.4)
	SCLC	68 (14.6)
	Other ^a	28 (6.0)
NSCLC	SCC	167 (35.8)
	ADC	204 (43.7)
Stage (NSCLC)	Early stage (I, II)	13 (2.8)
	Advanced stage (III, IV)	394 (84.4)
Stage (SCLC)	Limited stage	34 (7.3)
	Extensive stage	26 (5.6)
Chemotherapy regimen	GP	192 (41.1)
	EP	68 (14.6)
	PP	137 (29.3)
	TP	27 (5.8)
	DP	29 (6.2)
	Other ^b	14 (3.0)
Overall toxicity	Grade 0–2	286 (61.2)
	Grade 3–4	181 (38.8)
Performance score (PS)	0	42 (9.0)
	1	395 (84.6)
	2	30 (6.4)
Gastrointestinal toxicity	Grade 0–2	366 (78.4)
	Grade 3–4	101 (21.6)
Hematologic toxicity	Grade 0–2	353 (75.6)
	Grade 3–4	114 (24.4)

Abbreviations: NSCLC, non-small-cell lung cancer; SCC, squamous-cell carcinoma; ADC, adenocarcinoma; SCLC, small-cell lung cancer; Other^a, mixed-cell or undifferentiated carcinoma; GP, platinum+gemcitabine; EP, platinum+etoposide; PP, platinum+pemetrexed; TP, platinum+paclitaxel; DP, platinum+docetaxel; Other^b, platinum+irinotecan or platinum+navelbine; n, number.

lung cancer patients who received platinum-based chemotherapy. The median age was 57 years (range, 20–80 years), and 79.4% of the patients were male. Among these individuals, three hundred seventy-one patients were diagnosed as

NSCLC. Most of the patients were in the late stages. One hundred one (21.6%) and one hundred fourteen patients (24.4%) suffered from severe gastrointestinal and hematological toxicities, respectively. One hundred eighty-one patients (38.8%) experienced at least one type of severe toxicity (Table 1). Detailed information of the clinical characteristics of patients with gastrointestinal toxicity or hematologic toxicity is provided in Supplementary Table S1 and S2, respectively. Except for rs1012068, the call rates of the selected SNPs ranged from 96.1% to 100.0%. The genotype frequencies of SNPs were in Hardy-Weinberg equilibrium (Supplementary Table S3).

Association of lncRNA polymorphisms with severe overall toxicity

Unconditional logistic regression analysis was performed to reveal the association between well-characterized lung cancer lncRNA polymorphisms and severe overall toxicity. *ANRIL* rs1333049 was associated with the reduced incidence of severe overall toxicity in an additive model (adjusted OR=0.723, 95% CI=0.541–0.965, $P=0.028$) (Table 2). Subsequently, stratification analysis showed that *ANRIL* rs1333049 was associated with a low risk of severe overall toxicity in age ≥57 (additive model: OR=0.559, 95% CI=0.357–0.875, $P=0.011$; dominant model: OR=0.473, 95% CI=0.243–0.920, $P=0.027$), performance score (PS)=1 (additive model: OR=0.680, 95% CI=0.491–0.940, $P=0.020$; dominant model: OR=0.570, 95% CI=0.347–0.937, $P=0.027$), NSCLC (additive model: OR=0.681, 95% CI=0.489–0.949, $P=0.023$; dominant model: OR=0.578, 95% CI=0.349–0.959, $P=0.034$), and advanced NSCLC patients (additive model: OR=0.715, 95% CI=0.521–0.982, $P=0.038$). *ANRIL* rs10120688 was associated with an increased risk of overall toxicity in the recessive model (OR=2.308, 95% CI=1.126–4.734, $P=0.022$). *H19* rs2107425 was associated with a reduced incidence of severe overall toxicity in age <57 and adenocarcinoma (ADC) patients in a recessive model (OR=0.367, 95% CI=0.138–0.976, $P=0.045$; OR=0.232, 95% CI=0.063–0.853, $P=0.029$). However, for small cell lung cancer (SCLC) and patients treated with platinum+etoposide chemotherapy, patients with a C allele at *H19* rs2107425 showed increased risk of overall toxicity (OR=4.152, 95% CI=1.134–15.200, $P=0.032$; OR=4.239, 95% CI=1.245–14.430, $P=0.020$). *HOTAIR* rs7958904 was associated with a low incidence of severe overall toxicity in NSCLC and advanced NSCLC patients in a recessive model (OR=0.333, 95% CI=0.121–0.915, $P=0.033$; OR=0.306, 95% CI=0.110–0.854, $P=0.024$) and in those with PS=1 (additive model: OR=0.634, 95% CI=0.443–0.907, $P=0.013$; dominant model: OR=0.621, 95% CI=0.402–0.958, $P=0.031$) (Table 3).

Association of lncRNA polymorphisms with severe gastrointestinal toxicity

Logistic regression analysis revealed *ANRIL* rs1333049 was associated with a reduced incidence of severe gastrointestinal toxicity (additive model: OR=0.690, 95% CI=0.489–0.974, $P=0.035$; dominant model: OR=0.558, 95% CI=0.335–0.931, $P=0.025$), and *MEG3* rs116907618 was associated with an increased incidence of severe gastrointestinal toxicity in an additive model (OR=1.717, 95% CI=1.007–2.927, $P=0.047$)

(Table 2). Further stratification analyses showed that *ANRIL* rs1333049 was associated with a low risk of severe gastrointestinal toxicity in males (dominant model: OR=0.510, 95% CI=0.285–0.913, $P=0.023$), ADC (additive model: OR=0.520, 95% CI=0.291–0.928, $P=0.027$; recessive model: OR=0.279, 95% CI=0.090–0.864, $P=0.027$) and squamous cell carcinoma (SCC) (OR=0.327, 95% CI=0.137–0.784, $P=0.012$). *ANRIL* rs1133049 was also associated with reduced severe gastrointestinal toxicity in cisplatin-based chemotherapy, platinum+gemcitabine (GP) regimen, NSCLC, advanced NSCLC and smoking patients in both the additive (OR=0.625, 95% CI=0.425–0.919, $P=0.017$; OR=0.564, 95% CI=0.328–0.970, $P=0.038$; OR=0.599, 95% CI=0.399–0.899, $P=0.013$; OR=0.660, 95% CI=0.451–0.967, $P=0.033$; OR=0.622, 95% CI=0.394–0.982, $P=0.041$) and dominant (OR=0.544, 95% CI=0.308–0.963, $P=0.037$; OR=0.441, 95% CI=0.197–0.987, $P=0.046$; OR=0.475, 95% CI=0.264–0.855, $P=0.013$; OR=0.572, 95% CI=0.327–0.999, $P=0.050$; OR=0.428, 95% CI=0.221–0.828, $P=0.012$) models. *MEG3* rs116907618 was associated with high risk of severe gastrointestinal toxicity in NSCLC, SCC, GP and advanced NSCLC in both additive (OR=2.178, 95% CI=1.197–3.965, $P=0.011$; OR=3.538, 95% CI=1.269–9.864, $P=0.016$; OR=2.735, 95% CI=1.192–6.274, $P=0.018$; OR=2.118, 95% CI=1.196–3.753, $P=0.010$) and dominant (OR=2.195, 95% CI=1.135–4.242, $P=0.019$; OR=3.538, 95% CI=1.269–9.864, $P=0.016$; OR=2.695, 95% CI=1.131–6.421, $P=0.025$; OR=2.149, 95% CI=1.153–4.007, $P=0.025$) models. *H19* rs2107425 was associated with a low risk of severe gastrointestinal toxicity in age <57, ADC and NSCLC in a recessive model (OR=0.182, 95% CI=0.047–0.812, $P=0.026$; OR=0.124, 95% CI=0.015–0.995, $P=0.049$; OR=0.228, 95% CI=0.067–0.778, $P=0.018$) and associated with high risk in SCLC patients in a dominant model (OR=5.937, 95% CI=1.149–32.230, $P=0.039$). *H19* rs2839698 was associated with the increased incidence of severe gastrointestinal toxicity in age ≥ 57 (recessive model: OR=4.037, 95% CI=1.168–13.950, $P=0.027$) and female patients (additive model: OR=2.223, 95% CI=1.033–4.783, $P=0.041$; dominant model: OR=2.796, 95% CI=1.011–7.736, $P=0.048$). *MALAT1* rs619586 was associated with increased incidence of severe gastrointestinal toxicity in smoke and age ≥ 57 in both the additive (OR=1.978, 95% CI=1.052–3.721, $P=0.034$; OR=2.339, 95% CI=1.082–5.055, $P=0.031$) and dominant (OR=2.373, 95% CI=1.156–4.874, $P=0.019$; OR=3.005, 95% CI=1.295–6.971, $P=0.010$) models. *HOTAIR* rs1899663 was associated with a low risk of severe gastrointestinal toxicity in patients age >57 (dominant model: OR=0.369, 95% CI=0.157–0.868, $P=0.022$). *HOTAIR* rs7958904 was associated with a reduced incidence of severe gastrointestinal toxicity in SCC, cisplatin-based chemotherapy, NSCLC and advanced NSCLC in both the additive (OR=0.436, 95% CI=0.199–0.995, $P=0.038$; OR=0.514, 95% CI=0.333–0.793, $P=0.003$; OR=0.553, 95% CI=0.349–0.876, $P=0.011$; OR=0.608, 95% CI=0.395–0.937, $P=0.024$) and dominant (OR=0.404, 95% CI=0.167–0.978, $P=0.044$; OR=0.451, 95% CI=0.269–0.756, $P=0.003$; OR=0.525, 95% CI=0.303–0.908, $P=0.021$; OR=0.591, 95% CI=0.354–0.986, $P=0.044$) models. *POLR2E* rs3787016 was associated with

increased incidence of severe gastrointestinal toxicity in age ≥ 57 (additive model: OR=1.726, 95% CI=1.019–2.924, $P=0.042$; recessive model: OR=3.159, 95% CI=1.402–7.121, $P=0.006$), smoke (dominant model: OR=0.479, 95% CI=0.249–0.921, $P=0.027$) and male patients (recessive model: OR=2.147, 95% CI=1.196–3.853, $P=0.010$) (Table 4).

Association of lncRNA polymorphisms with severe hematologic toxicity

Logistic regression analysis did not reveal a significant association between lncRNA SNPs and severe hematologic toxicity. By subgroup analysis, *HOTTIP* rs5883064 was associated with increased incidence of severe hematological toxicity in nonsmokers (additive model: OR=1.973, 95% CI=1.101–3.533, $P=0.022$; recessive model: OR=3.807, 95% CI=1.327–10.920, $P=0.013$). *CCAT2* rs6983267 was associated with a reduced incidence of severe hematologic toxicity in NSCLC in both the additive (OR=0.614, 95% CI=0.423–0.891, $P=0.010$) and dominant (OR=0.522, 95% CI=0.312–0.875, $P=0.014$) models. *ANRIL* rs1333049 was associated with a low risk of severe hematological toxicity in patients age ≥ 57 in both the additive (OR=0.601, 95% CI=0.371–0.975, $P=0.039$) and recessive (OR=0.299, 95% CI=0.115–0.775, $P=0.013$) models. *ANRIL* rs10120688 was associated with an increased incidence of severe hematologic toxicity in smoking patients (dominant model: OR=1.858, 95% CI=1.024–3.371, $P=0.041$). Patients treated with GP regimen with AA genotype of *H19* rs2839698 showed a low risk of severe hematological toxicity (OR=0.120, 95% CI=0.015–0.962, $P=0.046$). *HOTAIR* rs7958904 was associated with an increased incidence of severe hematologic toxicity in SCC (additive model: OR=1.933, 95% CI=1.038–3.598, $P=0.038$; OR=3.794, 95% CI=1.679–8.572, $P=0.001$) and patients with GP regimen in a dominant model (OR=2.354, 95% CI=1.217–4.553, $P=0.011$). Patients with *POLR2E* rs3787016 A allele showed a low risk of severe hematologic toxicity with an age <57 (OR=0.498, 95% CI=0.251–0.990, $P=0.047$) (Table 5).

GMDR: gene-gene and gene-environment interactions and toxicity induced by platinum-based chemotherapy

GMDR was used to evaluate gene-gene interactions of 14 polymorphisms in 8 lncRNAs and overall, gastrointestinal, and hematological toxicities with adjustment for age, sex, smoking status, stage, PS, platinum dose, chemotherapy interval, preventive treatment, the time of examining the blood for hematotoxicity, histological type and chemotherapy regimens. The best predictive models of gene-gene interaction are presented in Supplementary Table S4. However, for each model, the interaction was not significant. For the gene-environment analysis, GMDR identified the three-factor interaction model of rs3787016-rs3807598-chemotherapy regimen as the best model for hematological toxicity, with a testing balance accuracy of 0.5902, and a maximum cross-validation consistency of 9/10 ($P=0.0107$) (Figure 1). However, other best predictive models of gene-environment interaction did not reach significance (Table 6).

Figure 1. The three-factor model of gene-environment interaction and hematological toxicity. The best model included a rs3787016-rs3807598-chemotherapy regimen. For each cell, the left bar represented a positive score, and the right bar indicated a negative score. High-risk cells are indicated in dark gray, and low-risk cells are indicated in light gray. The patterns of high-risk and low-risk cells differed across each of the different multi-locus dimensions, presenting evidence of epistasis.

Table 2. Association between lncRNA polymorphisms and platinum-based chemotherapy toxicity.

Type of toxicity	Gene	SNP	Genotype	Grade 0–2		Grade 3–4		Additive		Dominant		Recessive	
				n (%)	n (%)	n (%)	n (%)	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Overall toxicity	ANRIL	rs1333049	GG	69 (24.3)	32 (17.8)	0.723 (0.541, 0.965)	0.028*	0.662 (0.424, 1.035)	0.070	0.649 (0.399, 1.054)	0.080		
			GC	152 (53.5)	97 (53.9)								
			CC	63 (22.2)	51 (28.3)								
Gastrointestinal toxicity	ANRIL	rs1333049	GG	83 (22.9)	18 (17.8)	0.690 (0.489, 0.974)	0.035*	0.558 (0.335, 0.931)	0.025*	0.711 (0.396, 1.276)	0.252		
			GC	198 (54.5)	51 (50.5)								
			CC	82 (22.6)	32 (31.7)								
	MEG3	rs116907618	CC	1 (0.3)	2 (1.9)	1.717 (1.007, 2.927)	0.047*	1.678 (0.941, 2.992)	0.080	6.149 (0.540, 70.060)	0.143		
			CG	51 (13.9)	20 (19.8)								
			GG	314 (85.8)	79 (78.2)								

Abbreviations: SNP, single nucleotide polymorphism; n, number; OR, odd ratio; CI, confidence interval. *P<0.05.

Table 3. Stratification analysis of the association between lncRNA polymorphisms and overall toxicity.

Gene	SNP	Subgroup	Additive OR (95% CI)	P	Dominant OR (95% CI)	P	Recessive OR (95% CI)	P
ANRIL	rs1333049	More 57 ^a	0.559 (0.357, 0.875)	0.011 [*]	0.473 (0.243, 0.920)	0.027 [*]	0.475 (0.220, 1.025)	0.058
		NSCLC	0.681 (0.489, 0.948)	0.023 [*]	0.578 (0.349, 0.959)	0.034 [*]	0.667 (0.386, 1.151)	0.146
		Advanced NSCLC	0.715 (0.521, 0.982)	0.038 [*]	0.675 (0.416, 1.097)	0.112	0.615 (0.359, 1.054)	0.077
		PS 1	0.680 (0.491, 0.940)	0.020 [*]	0.570 (0.347, 0.937)	0.027 [*]	0.656 (0.382, 1.127)	0.126
H19	rs10120688	NSCLC	1.142 (0.809, 1.612)	0.450	0.899 (0.563, 1.435)	0.656	2.308 (1.126, 4.734)	0.022 [*]
		Less 57 ^b	0.871 (0.561, 1.368)	0.543	1.200 (0.660, 2.181)	0.550	0.367 (0.138, 0.976)	0.045 [*]
		ADC	0.734 (0.452, 1.195)	0.214	0.955 (0.498, 1.831)	0.889	0.232 (0.063, 0.853)	0.029 [*]
		SCLC	2.185 (0.886, 5.385)	0.089	4.152 (1.134, 15.200)	0.032 [*]	1.263 (0.236, 6.745)	0.785
HOTAIR	rs7958904	EP	2.100 (0.892, 4.943)	0.089	4.239 (1.245, 14.430)	0.020 [*]	0.993 (0.201, 4.899)	0.993
		NSCLC	0.779 (0.544, 1.116)	0.173	0.914 (0.586, 1.425)	0.690	0.333 (0.121, 0.915)	0.033 [*]
		Advanced NSCLC	0.804 (0.568, 1.136)	0.216	0.931 (0.609, 1.422)	0.740	0.306 (0.110, 0.854)	0.024 [*]
		PS 1	0.634 (0.443, 0.907)	0.013 [*]	0.621 (0.402, 0.958)	0.031 [*]	1.350 (0.173, 10.530)	0.775

Abbreviations: SNP, single nucleotide polymorphism; OR, odd ratio; CI, confidence interval; NSCLC, non-small-cell lung cancer; ADC, adenocarcinoma; SCLC, small-cell lung cancer; EP, platinum+etoposide; PS, performance score. More 57^a=age more 57. Less 57^b=age less 57. ^{*}P<0.05.

Table 4. Stratification analysis of the association between lncRNA polymorphisms and gastrointestinal toxicity.

Gene	SNP	Subgroup	Additive OR (95% CI)	P	Dominant OR (95% CI)	P	Recessive OR (95% CI)	P
ANRIL	rs1333049	Male	0.695 (0.466, 1.035)	0.073	0.510 (0.285, 0.913)	0.023 [*]	0.825 (0.422, 1.603)	0.566
		NSCLC	0.599 (0.399, 0.899)	0.013 [*]	0.475 (0.264, 0.855)	0.013 [*]	0.584 (0.288, 1.184)	0.136
		ADC	0.520 (0.291, 0.928)	0.027 [*]	0.564 (0.241, 1.320)	0.187	0.279 (0.090, 0.864)	0.027 [*]
		SCC	0.635 (0.344, 1.175)	0.148	0.327 (0.137, 0.784)	0.012 [*]	1.205 (0.443, 3.279)	0.715
		Cisplatin ^a	0.625 (0.425, 0.919)	0.017 [*]	0.544 (0.308, 0.963)	0.037 [*]	0.549 (0.280, 1.074)	0.080
		GP	0.564 (0.328, 0.970)	0.038 [*]	0.441 (0.197, 0.987)	0.046 [*]	0.518 (0.203, 1.322)	0.169
		Smoke	0.622 (0.394, 0.982)	0.041	0.428 (0.221, 0.828)	0.012 [*]	0.756 (0.355, 1.608)	0.467
		Advanced NSCLC	0.660 (0.451, 0.967)	0.033 [*]	0.572 (0.327, 0.999)	0.050 [*]	0.603 (0.308, 1.181)	0.140
H19	rs2107425	Less 57 ^b	0.874 (0.536, 1.426)	0.591	1.389 (0.712, 2.711)	0.336	0.182 (0.047, 0.812)	0.026 [*]
		ADC	0.673 (0.374, 1.208)	0.184	0.885 (0.411, 1.909)	0.756	0.124 (0.015, 0.995)	0.049 [*]
		NSCLC	0.790 (0.519, 1.201)	0.270	1.078 (0.610, 1.904)	0.796	0.228 (0.067, 0.778)	0.018 [*]
		SCLC	2.541 (0.847, 7.619)	0.096	5.937 (1.049, 32.230)	0.039 [*]	1.173 (0.154, 9.150)	0.879
MALAT1	rs2839698	More 57 ^c	1.483 (0.819, 2.687)	0.194	1.188 (0.555, 2.541)	0.657	4.037 (1.168, 13.950)	0.027 [*]
		Female	2.223 (1.033, 4.783)	0.041 [*]	2.796 (1.011, 7.736)	0.048 [*]	2.842 (0.544, 14.860)	0.216
		Smoke	1.978 (1.052, 3.721)	0.034 [*]	2.373 (1.156, 4.874)	0.019 [*]	1.234 (0.118, 12.910)	0.861
		More 57 ^c	2.339 (1.082, 5.055)	0.031 [*]	3.005 (1.295, 6.971)	0.010 [*]	2.230 (0.636, 20.750)	0.998
HOTAIR	rs1899663	More 57 ^c	0.501 (0.241, 1.042)	0.064	0.369 (0.157, 0.868)	0.022 [*]	1.164 (0.222, 6.095)	0.857
		NSCLC	0.553 (0.349, 0.876)	0.011 [*]	0.525 (0.303, 0.908)	0.021 [*]	0.341 (0.093, 1.238)	0.102
		SCC	0.436 (0.199, 0.955)	0.038 [*]	0.404 (0.167, 0.978)	0.044 [*]	0.306 (0.029, 3.226)	0.324
		Cisplatin ^a	0.514 (0.333, 0.793)	0.003 [*]	0.451 (0.269, 0.756)	0.003 [*]	0.429 (0.140, 1.320)	0.140
MEG3	rs7958904	Advanced NSCLC	0.608 (0.395, 0.937)	0.024 [*]	0.591 (0.354, 0.986)	0.044 [*]	0.375 (0.106, 1.329)	0.129
		NSCLC	2.178 (1.197, 3.965)	0.011 [*]	2.195 (1.135, 4.242)	0.019 [*]	7.351 (0.632, 85.500)	0.111
		SCC	3.538 (1.269, 9.864)	0.016 [*]	3.538 (1.269, 9.864)	0.016 [*]	3.684 (0.549, 10.269)	0.887
		GP	2.735 (1.192, 6.274)	0.018 [*]	2.695 (1.131, 6.421)	0.025 [*]	2.440 (0.789, 12.872)	0.989
POLR2E	rs116907618	Advanced NSCLC	2.118 (1.196, 3.753)	0.010 [*]	2.149 (1.153, 4.007)	0.016 [*]	6.193 (0.539, 71.190)	0.143
		More 57 ^c	1.726 (1.019, 2.924)	0.042 [*]	1.320 (0.560, 3.111)	0.526	3.159 (1.402, 7.121)	0.006 [*]
		Smoke	0.714 (0.466, 1.096)	0.124	0.479 (0.249, 0.921)	0.027 [*]	0.898 (0.442, 1.825)	0.766
		Male	1.430 (0.972, 2.105)	0.070	1.132 (0.603, 2.124)	0.699	2.147 (1.196, 3.853)	0.010 [*]

Abbreviations: SNP, single nucleotide polymorphism; OR, odd ratio; CI, confidence interval; NSCLC, non-small-cell lung cancer; ADC, adenocarcinoma; SCLC, small-cell lung cancer; SCC, squamous-cell carcinoma; GP, platinum+gemcitabine. Cisplatin^a, cisplatin-based chemotherapy. Less 57^b, age less 57. More 57^c, age more 57. ^{*}P<0.05.

Table 5. Stratification analysis of the association between lncRNA polymorphisms and hematologic toxicity.

Gene	SNP	Subgroup	Additive	<i>P</i>	Dominant	<i>P</i>	Recessive	<i>P</i>
			OR (95% CI)		OR (95% CI)		OR (95% CI)	
<i>ANRIL</i>	rs1333049	Male	0.695 (0.466, 1.035)	0.073	0.510 (0.285, 0.913)	0.023*	0.825 (0.422, 1.603)	0.566
<i>HOTTIP</i>	rs5883064	Non-smoke	1.973 (1.101, 3.533)	0.022*	1.857 (0.801, 4.306)	0.149	3.807 (1.327, 10.920)	0.013*
<i>CCAT2</i>	rs6983267	NSCLC	0.614 (0.423, 0.891)	0.010*	0.522 (0.312, 0.875)	0.014*	0.542 (0.262, 1.122)	0.099
<i>ANRIL</i>	rs1333049	More 57 ^a	0.601 (0.371, 0.975)	0.039*	0.734 (0.357, 1.509)	0.401	0.299 (0.115, 0.775)	0.013*
	rs10120688	Smoke	1.497 (0.978, 2.291)	0.063	1.858 (1.024, 3.371)	0.041*	1.394 (0.575, 3.382)	0.462
<i>H19</i>	rs2839698	GP	0.755 (0.455, 1.254)	0.278	0.963 (0.516, 1.799)	0.907	0.120 (0.015, 0.962)	0.046*
<i>HOTAIR</i>	rs7958904	SCC	1.933 (1.038, 3.598)	0.038*	3.794 (1.679, 8.572)	0.001*	0.184 (0.019, 1.758)	0.141
		GP	1.529 (0.904, 2.586)	0.113	2.354 (1.217, 4.553)	0.011*	0.337 (0.065, 1.736)	0.193
<i>POLR2E</i>	rs3787016	Less 57 ^b	0.659 (0.414, 1.049)	0.078	0.498 (0.251, 0.990)	0.047*	0.708 (0.319, 1.572)	0.396

Abbreviations: SNP, single nucleotide polymorphism; OR, odd ratio; CI, confidence interval; NSCLC, non-small-cell lung cancer; SCC, squamous-cell carcinoma; GP, platinum+gemcitabine. More 57^a, age more 57. Less 57^b, age less 57. **P*<0.05.

Table 6. Association of gene-environment interaction and toxicities induced by platinum-based chemotherapy.

Type of toxicity	Model	Training Bal Acc	Testing Bal Acc	Sign test (<i>P</i>)	CV consistency
Overall toxicity	Regimen	0.5983	0.5897	0.0107*	10/10
	rs6983267 regimen	0.6252	0.5168	0.6230	5/10
	rs10120688 rs6983267 regimen	0.6777	0.5044	0.3770	5/10
	Age	0.5938	0.5431	0.1719	6/10
Gastrointestinal toxicity	rs2107425 age	0.6319	0.5420	0.0547	9/10
	rs2107425 rs6983267 regimen	0.6868	0.5104	0.1719	5/10
	Regimen	0.6165	0.6005	0.0547	10/10
Hematological toxicity	rs2839698 regimen	0.6469	0.5155	0.3770	4/10
	rs3787016 rs3807598 regimen	0.7098	0.5902	0.0107*	9/10

Abbreviations: Training Bal Acc, training balance accuracy; Testing Bal Acc, testing balance accuracy. **P*<0.05.

Discussion

lncRNAs played important roles in various aspects of pathophysiological activities, such as carcinogenesis, drug resistance and toxicity. In previous studies, we explored the association of well-characterized lung cancer lncRNA genetic polymorphisms with lung cancer susceptibility and platinum-based chemotherapy response. In this study, we provided the first investigation into the association between lncRNA polymorphisms and severe toxicity induced by platinum-based chemotherapy in lung cancer patients. The results showed that *ANRIL* rs1333049 and rs10120688, *H19* rs2107425 and *HOTAIR* rs7958904 were associated with the incidence of severe overall toxicity. *ANRIL* rs1333049 and rs10120688, *H19* rs2107425 and rs2839698, *HOTAIR* rs1899663 and rs7958904, and *MEG3* rs116907618 were associated with the risk of severe gastrointestinal or hematologic toxicity. The three-factor interaction model of *POLR2E* rs3787016-*HOTTIP* rs3807598-chemotherapy regimen was the best predictive model for hematological toxicity.

ANRIL, transcribed from the INK4B-ARF-INK4A gene cluster, was overexpressed in NSCLC patient tissues and correlated with TNM stage and prognosis^[13]. The knockdown of

ANRIL expression impaired cell proliferation, migration and invasion, and induced cell apoptosis in esophageal cancer, gastric cancer, and lung cancer cell lines^[13, 14]. *ANRIL* controlled the epigenetic silencing of p14ARF and p16INK4a, which were important regulators of apoptosis induced by cisplatin^[15-17]. The results also revealed that *ANRIL* rs1333049 was associated with the incidence of severe gastrointestinal toxicity. Rs1333049, located at a hotspot region in genome wide association studies, was associated with various diseases and platinum-based chemotherapy response^[10]. *ANRIL* rs10120688 was associated with severe hematologic toxicity. Although those SNPs were not associated with *ANRIL* expression, these mutations might affect the centroid secondary structure and minimum free energy of *ANRIL*, thereby influencing the function of *ANRIL*^[10, 18].

H19, a paternally imprinted gene transcribed from chromosome 11p15, was maternally expressed during embryonic development, but postnatally inactivated in most tissues^[19]. The overexpression of *H19* was associated with the poor survival of lung cancer patients^[20]. The expression of *H19* could be induced by cigarette smoke condensate in human respiratory epithelial cells^[21]. As an oncogene, *H19* mediated cell pro-

liferation, metastasis, and angiogenesis. *H19* induced multi-drug resistance 1 (*MDR1*) expression and *MDR1*-associated drug resistance by regulating *MDR1* promoter methylation^[22]. *MDR1* was involved in gastrointestinal toxicity caused by platinum-based chemotherapy^[23]. According to the data in Genecards (<http://www.genecards.org>), *H19* was highly expressed in the bone marrow and stomach. Our results indicated that *H19* rs2104725 and rs2839698 were associated with severe gastrointestinal toxicity, and rs2839698 was associated with severe hematologic toxicity in patients received GP regimen. Previous studies have shown that these two SNPs were associated with platinum-based chemotherapy responses. Rs2107425 did not affect the expression of *H19*, but might alter its secondary structure^[10]. Rs2839698 was associated with serum *H19* mRNA levels^[24].

HOTAIR, transcribed from the human *HOXC* locus, was overexpressed in a plethora of cancerous tissues and associated with metastasis and prognosis^[25]. *HOTAIR* was upregulated in cisplatin-resistant A549 cells. The overexpression of *HOTAIR* could lead to the cisplatin resistance in A549 cells^[26]. We found that *HOTAIR* rs7958904 was associated with both severe gastrointestinal toxicity and hematologic toxicity. Previous studies showed that rs7958904 was associated with colorectal cancer risk and platinum-based chemotherapy responses in lung cancer and might affect the secondary structure of *HOTAIR*^[10, 27]. Rs1899663 was associated with severe gastrointestinal toxicity in age ≥ 57 . The SNP rs12826786 in strong linkage disequilibrium with rs1899663 ($r^2=1$) was asso-

ciated with *HOTAIR* expression^[28].

MEG3, located on human chromosome 14q32, was down-regulated in NSCLC tissues and correlated with pathological stage, tumor size, and prognosis^[29]. *MEG3* was decreased in cisplatin-resistant A549/DDP cells, and the overexpression of *MEG3* increased cisplatin sensitivity by inhibiting cell proliferation and inducing apoptosis^[30]. *MEG3* was overexpressed in the stomach. Our findings revealed that *MEG3* rs116907618 was associated with severe gastrointestinal toxicity. An RNA-fold web server predicted that the polymorphism of rs116907618 might alter the centroid secondary structure and minimum free energy of *MEG3*, changing the folding and function of *MEG3* (Figure 2A and 2B). However, this hypothesis needs thorough investigation.

MALAT1, transcribed from chromosome 11q13, was a predictive marker for metastasis and prognosis in several cancers, including lung cancer^[31]. The silencing of *MALAT1* remarkably increased the sensitivity of cancer cells to cisplatin^[32]. We observed that *MALAT1* rs619586 was associated with gastrointestinal toxicity. Previous studies have shown that rs619586 was associated with platinum-based chemotherapy responses and might change *MALAT1* expression by affecting the transcription factor binding sites^[10].

Platinum-based chemotherapy toxicities resulted from multiple genes and environmental factors, each of which might demonstrate a minor marginal effect. To further assess the combined effects, GMDR was used to explore gene-gene and gene-environment interactions. The three-factor interaction

Figure 2. The centroid secondary structure of lncRNA sequence. (A) Structure of *MEG3* containing the G allele of rs116907618. The minimum free energy (MFE) of the secondary structure was -105.10 kcal/mol. (B) The structure of *MEG3* containing the C allele of rs116907618. The MFE of the secondary structure was -58.81 kcal/mol. (C) Structure of *POLR2E* containing the C allele of rs3787016. The MFE of the secondary structure was -87.40 kcal/mol. (D) Structure of *POLR2E* containing the T allele of rs3787016. The MFE of the secondary structure was -103.80 kcal/mol.

model of *POLR2E* rs3787016-*HOTTIP* rs3807598-chemotherapy regimen was the best predictive model for hematological toxicity. Rs3787016 was predicted to change the centroid secondary structure and minimum free energy of *POLR2E* (Figure 2C and 2D), whereas rs3807598 might affect the transcript factor binding sites of *HOTTIP* (http://snpinfo.niehs.nih.gov/cgi-bin/snpinfo/tfbs.cgi?2_rs3807598). Studies have reported that the majority of lncRNAs were transcribed by RNA polymerase II^[33], the subunit of which could be affected by *POLR2E*^[34]. Chemotherapy regimens were differently distributed between grade 0–2 and grade 3–4 hematological toxicity patients and observed as the best one-factor model for hematological toxicity. The results of GMDR indicated that the lncRNA *POLR2E*-*HOTTIP*-chemotherapy regimen may interact and affect hematological toxicity. However, the mechanism remains unknown.

Admittedly, the present study had some limitations. First, after FDR-BH correction at the 0.05 level^[35], rare SNPs remained significant, and the present study was conducted on a relatively small sample and should be verified on a large scale. Moreover, the functions of lncRNAs have not been widely explored. The precise mechanisms of the lncRNA SNPs in platinum-based chemotherapy toxicity remain unknown. Further studies are greatly needed. Additionally, the toxicities induced by platinum-based chemotherapy may result from multiple genetic factors. Thus, additional studies on different levels are needed.

In conclusion, *ANRIL* rs1333049 and rs10120688, *H19* rs2107425 and rs2839698, *HOTAIR* rs1899663 and rs7958904, and *MEG3* rs116907618 were associated with the incidence of severe gastrointestinal or hematologic toxicities. The three-factor interaction model of *POLR2E* rs3787016-*HOTTIP* rs3807598-chemotherapy regimen was the best predictive model for hematological toxicity, and these SNPs might be considered as potential predictive markers for platinum-based chemotherapy toxicity in Chinese lung cancer patients.

Acknowledgements

The authors would like to thank all of the patients who participated in the study. This work was supported by the National Key Research and Development Plan (SQ2016YFSF110100 and 2016YFC0905000), National Natural Science Foundation of China (81373490, 81573508, and 81573463), Hunan Provincial Science and Technology Plan of China (2015JC3025), and Open Foundation of Innovative Platform in University of Hunan Province of China (421530004).

Author contribution

Wei-jing GONG and Zhao-qian LIU conceived and designed the experiments; Wei-jing GONG, Ji-ye YIN, Xiang-ping LI, Wei ZHENG, Ling XIAO, Li-ming TAN, and Jing-bo PENG performed the experiments; Wei-jing GONG, Di XIAO, and Xi LI analyzed the data; Yi-xin CHEN, Hong-hao ZHOU, and Zhao-qian LIU contributed reagents/materials/analysis tools; Wei-jing GONG and Zhao-qian LIU drafted the manuscript.

Supplementary information

Supplementary information is available at the website of Acta Pharmacologica Sinica.

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