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Hsa-miR-196a2 Functional SNP is Associated With Severe Toxicity After Platinum-Based Chemotherapy of Advanced Nonsmall Cell Lung Cancer Patients in a Chinese Population

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Background: Rs11614913 is a polymorphism in *hsa-miR-196a2* reported to alter mature microRNA expression and function. This single-nucleotide polymorphism (SNP) was reported to be associated with susceptibility and prognosis of lung cancer. *Methods:* In this article, association study was performed to reveal the relation between SNP and response rate or severe toxicity after platinum-based regimen in advanced nonsmall cell lung cancer patients. *Results:* By screening this polymorphism in 442 Chinese patients with MALDI-TOF Mass Spectrometer, significantly higher occurrence of grade 3 or 4 overall toxicity ($P = 0.02$) in response to treatment was found in patients with homozygous CC. After stratified analyses, association between *rs11614912* and overall toxicity existed, especially in individuals treated with gemcitabine ($P = 0.006$) or cisplatin ($P = 0.008$), and in male patients ($P = 0.02$) or younger patients ($P = 0.01$). *Conclusion:* Our study confirmed that *rs11614913* in *hsa-miR-196a2* was associated with severe toxicity in lung cancer patients, and might help to improve individualized therapy in the future. J. Clin. Lab. Anal. 26:441–446, 2012.

Key words: microRNA; *rs11614913*; cisplatin; carboplatin; chemotherapeutics; prognosis; side effect

INTRODUCTION

Lung cancer continuously has the highest mortality among all types of cancer worldwide (1). Furthermore, nonsmall cell lung cancer (NSCLC) accounts for approximately 85% of the incidence. For NSCLC, the standard chemotreatment includes a platinum regimen and another cytotoxic agent (2, 3). However, they may bring low objective response rate (ORR) or severe toxicity in some patients that cause unsatisfactory prognosis.

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Thus, prognostic markers are required for optimal individualized therapy.

MicroRNAs (miRNAs), an abundant class of endogenous small noncoding RNAs, function as negative regulators by causing posttranscriptional gene silencing (4–6). In lung cancer, miRNA expression profiles and specific miRNAs have been proved to be correlated with survival, which indicates that miRNA expression profiles are diagnostic and prognostic markers of lung cancer (7). Moreover, single-nucleotide polymorphisms (SNPs) in miRNA genes could change the property of miRNAs through altering miRNA expression (8,9). Therefore, SNPs in miRNAs may also have functions and be applied as biomarkers for cancer prognosis. *Rs11614913*, one of the few SNPs in mature sequence of miRNAs (10), has this potential as it was reported that its variant homozygote CC had associations with significantly increased risk of lung, breast, pancreatic cancer, colorectal cancer, gastric cancer, and poor NSCLC survival (10–16). Hu et al. suggested that the SNP might affect mature *miR-196a* expression and binding of mature *hsa-miR-196a2–3p* to its target mRNA (10). So the correlations between *hsa-miR-196a2 rs11614913* and ORR and toxic severity of treatment in advanced NSCLC patients after platinum-based chemotherapy are worth exploring, since they are quite important factors in clinical practice.

MATERIALS AND METHODS

Patient Recruitment and Follow-up

In this study, we recruited 445 patients diagnosed with NSCLC at the Chest Hospital in Shanghai, China, between March 2005 and August 2008. All of them were inoperable, in stage IIIA-IV, and given first-line platinum-based chemotherapy. Clinical data were systematically recorded at admission. The incidence of grade 3 or 4 toxicity was assessed twice a week, according to the National Cancer Institute Common Toxicity Criteria version 3.0 (http://ctep.cancer.gov). ORR was evaluated after first two courses of chemotherapy using Response Evaluation Criteria in Solid Tumors (RECIST) guidelines. The details of patient recruitment and collection of clinical data can be consulted in our previous study (17). The investigators were blinded to the polymorphism status of the patients. The study protocol was approved by the Ethical Review Committee of the hospital.

Chemotherapy Regimens

All the 445 patients enrolled in the study were given first-line platinum-based chemotherapy (Navelbine: 25 mg/m^2, day 1 and day 8 every 3 weeks in combination with cisplatin 75 mg/m^2 or carboplatin AUC 5, both administered on day 1, every 3 weeks; Gemcitabine 1,250 mg/m^2, days 1 and 8 every 3 weeks in combination with cisplatin 75 mg/m^2 or carboplatin AUC 5, both administered on day 1, every 3 weeks; Taxol 175 mg/m^2, day 1 every 3 weeks in combination with cisplatin 75 mg/m^2 or carboplatin AUC 5, both administered on day 1, every 3 weeks; Docetaxel 75 mg/m^2, day 1 every 3 weeks in combination with cisplatin 75 mg/m^2, also administered on day 1, every 3 weeks). All chemotherapy drugs were administered intravenously and patients were treated for two to six cycles.

Hsa-miR-196a2 Genotyping

Blood samples were collected when patients registered themselves. The genomic DNA was extracted from peripheral leukocytes using QIAamp DNA Maxi Kit (Qiagen GmbH, Hilden, Germany). Four hundred and forty-two (99.3%) of the study subjects were successfully genotyped with *hsa-miR-196a2 rs11614913* through MassArray (Sequenom, San Diego, CA) by allele-specific MALDI-TOF mass spectrometry (18). A 5% random sample was tested in duplicate by different persons, and the reproducibility was 100%. All the results were generated and analyzed by laboratory staff unaware of patient status.

Statistical Analysis

Toxicity outcomes were dichotomized by the presence or absence of grade 3 or 4 toxicity, and ORR results were divided by the presence or absence of stable disease (SD) or progressive disease (PD). Severe overall toxicity required presence of severe hematologic toxicity (leukocytopenia, neutropenia, anemia, or thrombocytopenia) or gastrointestinal toxicity (nausea or vomiting). The associations between the polymorphism and low ORR or severe toxicity were estimated by odds ratios (OR) and their 95% confidence intervals (CI), which were calculated by unconditional logistic regression. All statistical analyses used SPSS, version 12.0. All *P* values reported were two-sided, and a level of 0.05 was regarded as statistical significance.

RESULTS

Patient Characteristics and Clinical Outcomes

Four hundred and forty-five patients including 317 (71.2%) male patients were histologically diagnosed as NSCLC (stage III or IV), in which adenocarcinoma represented the majority share up to 269 individuals (60.5%). There were 43 patients (9.7%) with stage IIIA, 131 (29.4%) with stage IIIB, and 271 (60.9%) with stage IV disease. The average age of subjects at diagnosis was 57 years old

TABLE 1. Clinical Characteristics of NSCLC Patients

Patients characteristics	Stratification	*n* (%)	Patients characteristics	Stratification	*n* (%)
Total no. of patients		445	Gender		
Median age (range)	57 (32–80)			Male	317 (71.2)
	≤57	226 (50.8)		Female	128 (28.8)
	>57	219 (49.2)	TNM stage		
PS				IIIA	43 (9.7)
	0–1	427 (96.0)		IIIB	131 (29.4)
	2	17 (4.0)		IV	271 (60.9)
Anemia			Chemotherapy regimen		
	Grade 1 or 2	391 (87.9)		Platinum-navelbine	233 (52.4)
	Grade 3 or 4	22 (4.9)		Platinum-gemcitabine	77 (17.3)
Thrombocytopenia				Platinum-paclitaxel	90 (20.2)
	Grade 1 or 2	392 (88.1)		Platinum-docetaxel	22 (4.9)
	Grade 3 or 4	26 (5.8)		Other platinum combinations	23 (5.2)
ORR			Platinum-based drug		
	CR or PR	76 (17.1)		Cisplatin	326 (73.3)
	SD or PD	342 (76.9)		Carboplatin	119 (26.7)
Leukocytopenia			Histological type		
	Grade 1 or 2	326 (73.3)		Adenocarcinoma	269 (60.5)
	Grade 3 or 4	104 (23.4)		Squamous cell	88 (19.8)
Neutropenia				Adenosquamocarcinoma	16 (3.6)
	Grade 1 or 2	340 (76.4)		Others[a]	72 (16.2)
	Grade 3 or 4	65 (14.6)	Overall toxicity		
Hematologic toxicity				Grade 1 or 2	240 (53.9)
	Grade 1 or 2	273 (61.4)		Grade 3 or 4	142 (31.9)
	Grade 3 or 4	129 (29.0)	*rs11614913*		
Gastrointestinal toxicity				CC	134 (30.1)
	Grade 1 or 2	376 (84.5)		CT	225 (50.6)
	Grade 3 or 4	27 (6.1)		TT	83 (18.7)

[a]Other carcinomas include mixed cell, neuroendocrine carcinoma, or undifferentiated carcinoma.
NSCLC, nonsmall cell lung cancer; TNM, tumor/node/metastasis; PS, performance status; ORR, objective response rate; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

(from 32 to 80 years old). Platinum-based chemotherapy was received as first line treatment in all subjects, among which 326 (73.3%) patients were treated with cisplatin and 233 (52.4%) received regimen of navelbine plus a platinum compound.

Response rate and all chemotherapy-related toxicities were recorded for each treatment cycle. Among total 445 patients, response rates were acquired from 418 individuals, hematologic and gastrointestinal toxicities were evaluated in 402 and 403 subjects, respectively. There were 142 (31.9%) patients suffered from grade 3 or 4 overall toxicity.

Hsa-miR-196a2 rs11614913 were successfully genotyped in 442 (99.3%) subjects. The numbers of patients with variant homozygous CC genotype, heterozygous CT, and homozygotes TT were 134 (30.2%), 227 (51.1%), and 83 (18.7%), respectively. The genotype distributions were under Hardy-Weinberg equilibrium ($P > 0.05$). Clinical and pathological characteristics of patients along with their genotypes of *rs11614913* are shown in Table 1.

Association Between *hsa-miR-196a2* Polymorphism and Clinical Outcomes

Association analyses between clinical outcomes and genotypes or alleles were performed to reveal the influence of the SNP on toxicities and response rate of treatment. After calculation, we found that *hsa-miR-196a2 rs11614913* had no significant association with leukocytopenia, neutropenia, anemia, thrombocytopenia, or hematologic toxicity (P values for TT/CT vs. CC were 0.35, 0.18, 0.96, 0.57, and 0.23, respectively) (Table 2). Neither did it have significant correlation with gastrointestinal toxicity, as the P value was 0.21 (TT/CT vs. CC) (Table 2). However, risk of severe overall toxicity was significantly higher in individuals with homozygous CC of *rs11614913* ($P = 0.02$; OR = 1.73; 95% CI 1.10–2.71), compared with T allele carriers (TT/CT). This association was no longer significant when TT was compared with CC/CT ($P = 0.26$; OR = 0.7; 95% CI 0.38–1.29) or T allele was compared with C allele ($P = 0.13$; OR = 0.79; 95% CI = 0.59–1.07). This suggested a recessive effect of C allele on overall toxicity. Because our patients

TABLE 2. Response and Toxicity Outcomes

Selected variables	*rs11614913*	Group 1[a] *n* (%)	Group 2[b] *n* (%)	*P*	OR (95% CI)[c]
Response					
	CT+TT	51 (11.5)	240 (54.3)		1.00 (reference)
	CC	23 (5.2)	101 (22.9)	0.66	0.88 (0.50–1.55)
Leukocytopenia					
	CT+TT	228 (51.6)	69 (15.6)		1.00 (reference)
	CC	95 (21.5)	35 (7.9)	0.35	1.25 (0.78–2.02)
Neutropenia					
	CT+TT	235 (53.2)	41 (9.3)		1.00 (reference)
	CC	102 (23.1)	24 (5.4)	0.18	1.48 (0.84–2.61)
Anemia					
	CT+TT	265 (60.0)	15 (3.4)		1.00 (reference)
	CC	123 (27.8)	7 (1.6)	0.96	1.00 (0.39–2.57)
Thrombocytopenia					
	CT+TT	269 (60.9)	16 (3.6)		1.00 (reference)
	CC	120 (27.2)	10 (2.3)	0.57	1.27 (0.55–2.92)
Hematologic toxicity					
	CT+TT	189 (42.8)	83 (18.8)		1.00 (reference)
	CC	81 (18.3)	46 (10.4)	0.23	1.32 (0.84–2.01)
Gastrointestinal toxicity					
	CT+TT	262 (59.3)	16 (3.6)		1.00 (reference)
	CC	111 (25.1)	11 (2.5)	0.21	1.72 (0.75–3.95)
Overall toxicity					
	CT+TT	176 (39.6)	88 (19.8)		1.00 (reference)
	CC	63 (14.3)	54 (12.2)	0.02[d]	1.73 (1.10–2.71)

[a]Group 1 for response means complete response or partial response, for toxicities means any grade 1 or 2 toxicities.
[b]Group 2 for response means stable disease or progressive disease, for toxicities means any grade 3 or 4 toxicities.
[c]Data were calculated by unconditional logistic regression, adjusting covariates for (i) response were age, gender, type of treatment regimen, tumor/node/metastasis (TNM) stage, performance status (PS), and histological type, (ii) anemia were PS, type of treatment regimen, and TNM stag, as TNM was a potential confounder for anemia, (iii) gastrointestinal toxicity were PS, gender, and type of treatment regimen, as gender was a potential confounder for gastrointestinal toxicity, (iv) other toxicities were PS and type of treatment regimen.
[d]Data of statistical significance.
OR, odds ratios; CI, confidence intervals.

were all inoperable, we also investigated the association between the SNP and ORR, which evaluates whether the drugs had brought enough effects to tumor cells. However, no significant correlation between them was observed (TT/CT vs. CC, $P = 0.66$; OR = 0.88; 95% CI 0.50–1.55) (Table 2).

Because different drugs could bring different side effects, we analyzed whether *hsa-miR-196a2 rs11614913* had significant association with any specific treatment. Among the patients receiving cisplatin regimen, the occurrence of severe overall toxicity was significantly higher in ones with homozygous CC of *rs11614913*, compared with T allele carriers (TT/CT) ($P = 0.008$; OR = 2.04; 95% CI 1.20–3.48). Meanwhile, the genotype CC raised the risk for 4.46-folds of grade 3 or 4 overall toxicity in individuals treaded with gemcitabine plus a platinum compound ($P = 0.006$; OR = 4.46; 95% CI 1.55–12.82). The SNP had no significant association with toxicities or ORR in any other treatments. Moreover, some factors beside genotype might also influence the result. Hence, the analyses were performed in subpopulation stratified by potential confounders such as gender and age. Compared with T allele carriers, subjects with the genotype CC might be more possible to experience severe toxicity if they were male ($P = 0.02$; OR = 1.92; 95% CI 1.13–3.25) or not older than 57 years ($P = 0.01$; OR = 2.23; 95% CI 1.91–4.16) (Table 3).

DISCUSSION

In agreement with the previous study concerning NSCLC survival (10), our results revealed that *rs11614913* in *hsa-miR-196a2* was significantly associated with prognosis of NSCLC, genotype CC of this polymorphism showed an unfavorable effect on overall toxicity but not ORR, especially in cisplatin-treated or platinum-gemcitabine-treated patients, which could offer pharmacogenomic reference to clinicians for optimal individualized therapy.

The function of *hsa-miR-196a* in pathogenesis of tumor has been largely investigated. Most studies on *hsa-miR-196a* suggest its oncogenic function in cancers (19).

TABLE 3. Stratification Analysis of Associations between *rs11614913* Genotypes and Severe Overall Toxicity by Selected Variables

Selected variables	*rs11614913*	Group 1[a]	Group 2[b]	*P*	OR (95% CI)[c]
Chemotherapy regimens					
Platinum-navelbine	CT+TT	87	55		1.00 (reference)
	CC	24	27	0.07	1.81(0.94–3.48)
Platinum-gemcitabine	CT+TT	35	10		1.00 (reference)
	CC	11	14	0.006[d]	4.46 (1.55–12.82)
Platinum-paclitaxel	CT+TT	43	13		1.00 (reference)
	CC	21	4	0.54	0.68 (0.20–2.36)
Platinum-based drug					
Cisplatin	CT+TT	113	68		1.00 (reference)
	CC	40	48	0.008[d]	2.04 (1.20–3.48)
Carboplatin	CT+TT	61	20		1.00 (reference)
	CC	23	6	0.74	0.84 (0.30–2.37)
Gender					
Male	CT+TT	124	60		1.00 (reference)
	CC	47	41	0.02[d]	1.92 (1.13–3.25)
Female	CT+TT	50	28		1.00 (reference)
	CC	16	13	0.42	1.46 (0.60–3.57)
Age					
≤57	CT+TT	90	36		1.00 (reference)
	CC	36	31	0.01[d]	2.23 (1.91–4.16)
>57	CT+TT	84	52		1.00 (reference)
	CC	27	23	0.36	1.39 (0.71–2.69)
TNM stage					
III	CT+TT	75	29		1.00 (reference)
	CC	28	19	0.09	1.88 (0.90–3.94)
IV	CT+TT	100	59		1.00 (reference)
	CC	35	35	0.08	1.68 (0.94–2.98)
Histological types					
Adenocarcinoma	CT+TT	104	53		1.00 (reference)
	CC	44	34	0.15	1.54 (0.87–2.70)
Squamous cell	CT+TT	33	18		1.00 (reference)
	CC	10	10	0.23	1.93 (0.66–5.63)

[a]Group 1 means any grade 1 or 2 overall toxicity.
[b]Group 2 means any grade 3 or 4 overall toxicity.
[c]Data were calculated by unconditional logistic regression, adjusting covariates were performance status and type of treatment regimen.
[d]Data of statistical significance.
OR, odds ratios; CI, confidence intervals; TNM, tumor/node/metastasis.

Higher level of *miR-196a* was found in pancreatic cancer, leukemia, and esophageal adenocarcinoma, negatively associated with survival (20–24). Moreover, CC genotype of *rs11614913* in *hsa-miR-196a2* increased the binding efficiency to its target mRNA (10). The presence of the CC genotype correlated with increased susceptibility to multiple types of cancer including lung cancer and gastric cancer, and in multiple human populations including Chinese and Turkish (10–16, 25, 26). Meanwhile, the opposite relationship has been described in glioma in our previously reported study, where the CC genotype was associated with a decreased risk (27). In the investigations of tumor prognosis, opposite results have also been reported. Homozygous C allele carriers had reduced survival time in lung cancer in Chinese patients (10) but survived longer in head and neck cancer in American patients (25). Our results confirmed the unfavorable effect in the treatment of Chinese lung cancer patients. Interestingly, the "variant" homozygote CC genotype as described in Chinese population studies is actually the dominant genotype in Caucasians. These discrepancies highlight the need for further investigation of this SNP in different tumors and patient populations.

By targeting several regulation molecules including HOXB8, HMGA2, and annexin A1, *miR-196a* may play critical roles in not only cancer pathogenesis but also in normal development (10,12,28,29). Moreover, the expression of *hsa-miR-196a* not only in tumor but also in normal cells was significantly altered by *rs11614913* in both tumor and normal cells (10). Our results of the significant association between overall toxicity and this SNP confirmed its effect in normal cells. Although not significant, for most toxicities, including leukocytopenia, neutropenia, thrombocytopenia, hematologic toxicity, and gastrointestinal

toxicity, CC genotype was more presented in severe toxicity groups. This might imply that in advanced NSCLC patients, the disparity in expression of *hsa-miR-196a* because the SNP similarly influences the sensitivity to platinating agents in hematopoietic and epithelial cells rather than tumor cells. And these hypotheses need further experiments to validate.

Although the number of patients in our study was not large enough to achieve a good statistical power (powers are between 0.65 and 0.85 in all observed significant associations), our subjects were all recruited from the same hospital, diagnosed with advanced NSCLC, and consistently treated with platinum anticancerogen without surgery. The relatively homogeneous therapeutic standard limits the potential confounding effect of difference across various hospitals. However, the SNP had no significant association with the hematologic or gastrointestinal toxicity. And the female patients were much fewer than the male patients. Further studies with larger group of study subjects are warranted to address these questions.

To the best of our knowledge, this is the first study demonstrating that *hsa-miR-196a2 rs11614913* is associated with severe overall toxicity in advanced NSCLC patients treated with platinum-based chemotherapy. Although it is still hypothetical, our study together with other reports had indicated that the SNP might have some link with NSCLC chemotherapic prognosis. Therefore, it might become a biomarker applied to limit the incidence and severity of toxicities in the future and improve quality of life in NSCLC patients after platinum-based chemotherapy.

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