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ORIGINAL ARTICLE

The T393C polymorphism of *GNAS1* as a predictor for chemotherapy sensitivity and survival in advanced non-small-cell lung cancer patients treated with gemcitabine plus platinum

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Abstract
Purpose The *GNAS1* gene is linked to proapoptotic signaling and correlates closely with clinical outcomes in many human cancers. The aim of this study was to evaluate whether the T393C polymorphism of the *GNAS1* gene could be used as a chemotherapy sensitivity and prognosis predictive marker of advanced non-small-cell lung cancer (NSCLC) treated with gemcitabine plus platinum (GP).
Methods In this study, we performed the PCR-restriction fragment length polymorphism assay to examine the genotypes of the *GNAS1* T393C polymorphism in 131 peripheral blood DNA specimens from advanced NSCLC patients with GP treatment.
Results The frequencies of the CC, CT, and TT genotypes in 131 advanced NSCLC cases were 25.2, 47.4, and 26.7%, respectively. The favorable TT genotype was significantly correlated with better overall survival (OS; $P < 0.05$) and longer progress-free survival (PFS; $P < 0.05$) compared with the CT or CC genotype. In the multivariate Cox proportional hazards model, the *GNAS1* T393C polymorphism was independently associated with overall survival after adjusting the clinicopathological factors ($P < 0.05$).
Conclusions This study suggests that the TT genotype of the *GNAS1* T393C polymorphism could be an independent prognostic marker to predict chemotherapy sensitivity, favorable OS and PFS in advanced NSCLC patients with GP treatment.

Keywords GNAS1 · Polymorphism · Advanced NSCLC · Chemotherapy · Prognosis

Introduction

Lung cancer is the leading cause of cancer-related mortality, of which non-small-cell lung cancer (NSCLC) accounts for approximately 85% [1]. Although surgery can be curative at the early stages of NSCLC, the majority of patients with NSCLC have advanced and metastatic disease, which makes it is not amenable to curative resection at diagnosis. The combination of gemcitabine and platinum (cisplatin or carboplatin) is one of the feasible and effective first line treatments for patients with advanced disease and has showed improved overall survival [2]. One of the most important mechanisms for anti-cancer drugs is induction of apoptosis in cancer cells. However, patients with similar clinical characteristics are different with response to platinum-based chemotherapy, the variant reactions may be affected by individual gene polymorphism of patient.

The Gs protein α subunit (GNAS1) gene has been found to play an important role in promoting tumor cells apoptosis

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[3]. There is increasing evidence that a common synonymous single nucleotide polymorphism (SNP) in exon 5 (T393C) of the *GNAS1* gene is associated with the prognosis of human malignancies [3–7]. Frey et al. [8] have showed the genotypes of the *GNAS1* T393C polymorphism are associated with altered Gαs expression. Down-regulation of Gαs could weaken the function of Gαs-dependent apoptotic pathway and suppress chemotherapy-induced cancer cell death, resulting in faster cancer progression and shorter patients' survival which may make the anti-cancer drugs less effective. Until now, the polymorphism state of the *GNAS1* T393C in NSCLC, as well as its association with the chemotherapy sensitivity and prognostic significance, has not yet been investigated.

In this study, we first conducted a single-hospital-based respective analysis of 131 patients with advanced NSCLC to elucidate a potential correlation between the T393C genotypes and the clinical outcomes of NSCLC patients after platinum-based doubled chemotherapy. Our results suggest that the TT genotype of the *GNAS1* T393C polymorphism was significantly associated with chemotherapy sensitivity and better survival of advanced NSCLC. Thus, the T393C polymorphism could serve as an useful predictive marker in guiding the physician for the selection of an optimal treatment regimen.

Patients and methods

Patients and clinical samples

We collected peripheral blood samples from patients diagnosed with NSCLC pathologically prior to chemotherapy at the Zhejiang Province Cancer Hospital (Zhejiang, China) between January 2008 and December 2010. Patients were enrolled in this study according following criteria: (1) histologically or cytologically confirmed NSCLC; (2) stage IIIB or IV disease; (3) age no more than 72; (4) Eastern Cooperative Oncology Group PS of 2 or less; (5) one or more measurable or assessable lesions; (6) life expectancy of more than 4 weeks; and (7) none of the patient had received chemotherapy or radiotherapy previously. Patients were excluded if they had symptomatic brain metastasis, malignancies other than NSCLC within the last 5 years.

Tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST), and all responses were evaluated at least 6 weeks after initial assessment, that is, complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) on the basis of change in lesion size derived from CT scans. Written informed consent was obtained from all patients enrolled in this study. The study has been approved by the Ethics and Scientific Committees of Zhejiang Province Cancer Hospital.

Chemotherapy regimens

Patients with NSCLC have received one of the following chemotherapy treatments: carboplatin AUC 5 mg/mL min on day 1 plus gemcitabine 1,000 mg/m^2 on days 1 and 8 every 3 weeks, or carboplatin was replaced with cisplatin 75 mg/m^2 on day 1. Each treatment was repeated for two to six cycles, unless unacceptable toxicity, disease progression or patients' refusal to continue treatment.

DNA extraction and genotyping

For the genotyping of T393C polymorphism, peripheral blood genomic DNA from patients was extracted using Qiagen blood mini kit (Qiagen, Germany) according to the manufacturer's protocol. Genomic DNA was subjected to PCR for 30 cycles of amplification with the following pair of primers: Fw: 5′-CTCCTAACTGACATGGTGCAA-3′ and Rv: 5′- TAA GGCCACACAAGTCGGGGT-3′. The 345 bp PCR products were digested using the restriction enzyme FokI and analyzed on a 2% agarose gel. The unrestricted products (345 bp) represent the TT genotype; the completely restricted products (259 and 86 bp) represent the CC genotype.

Statistical analysis

Statistical analysis was performed with the SPSS standard version 16.0 (SPSS Inc., Chicago, IL). Survival curves and the univariate analysis were analyzed by the Kaplan–Meier method. The prognostic value of different variables for clinical outcomes was estimated by multivariate analysis using the Cox regression model. The association between the *GNAS1* T393C polymorphism and clinicopathological characteristics was assessed by chi-square test. Differences were considered significant when $P < 0.05$.

Results

Patient characteristics and efficacy of treatment

A total of 131 patients with advanced NSCLC were recruited into this study, of which 88 were men and 33 were women. The mean age of patients was 54 ± 10.4 years (ranging from 25 to 72 years old). All patients were native-Chinese of Asian ethnicity. Staging was based upon the 7th edition of AJCC tumor-node-metastasis (TNM) staging system. There were 55 (42.0%)never smokers and 76 (58%) smokers. Of the 131 patients, 25 (19.1%) had Stage IIIB disease and 106 (80.9%) had stage IV disease. None of the patients had CR, while 40 patients had PR, 66 had SD, and 25 had PD. The median progress-free survival (PFS) was 5.70 months (95% CI, range 5.11–6.29 months), while

Table 1 Association between the *GNAS1* T393C genotypes and clinical characteristics of patients with advanced NSCLC ($n = 131$)

Characteristics	All	*GNAS1* T393C genotype			*P*
		CC	CT	TT	
Age (years)					
<60 (*n*, %)	72 (55.0)	18 (25.0)	35 (48.6)	19 (26.4)	
≥60 (*n*, %)	59 (45.0)	15 (25.4)	28 (47.5)	16 (27.1)	0.99
Sex					
Male (*n*, %)	88 (67.2)	22 (25.0)	45 (51.1)	21 (23.9)	
Female (*n*, %)	43 (32.8)	11 (25.6)	18 (41.9)	14 (32.5)	0.51
Smoking					
No (*n*, %)	55 (42.0)	16 (29.1)	24 (43.6)	15 (27.3)	
Yes (*n*, %)	76 (58.0)	17 (22.4)	39 (51.3)	20 (26.3)	0.61
ECOG					
0–1 (*n*, %)	107 (81.7)	27 (25.2)	50 (46.7)	30 (28.0)	
2 (*n*, %)	24 (18.3)	6 (25.0)	13 (54.2)	5 (20.8)	0.74
Stage					
IIIB (*n*, %)	25 (19.1)	6 (24.0)	10 (40.0)	9 (36.0)	
IV (*n*, %)	106 (80.9)	27 (25.5)	53 (50.0)	26 (24.5)	0.49
Histology					
Adeno (*n*, %)	67 (51.1)	16 (23.9)	33 (49.2)	18 (26.9)	
SCC (*n*, %)	46 (35.1)	12 (26.1)	23 (50.0)	11 (23.9)	0.92
Other (*n*, %)	18 (13.7)	5 (27.8)	7 (38.9)	6 (33.3)	
Chemotherapy					
Gem + Cisplatin (*n*, %)	83 (63.4)	21 (25.3)	38 (45.8)	24 (28.9)	
Gem + Carbo (*n*, %)	48 (36.6)	12 (25.0)	25 (52.1)	11 (22.9)	0.72

ECOG Eastern Cooperative Oncology Group, *Adeno* adenocarcinoma, *SCC* squamous cell carcinoma, *Gem + Cisplatin* Gemcitabine plus Cisplatin, *Gem + Carbo* gemcitabine plus carboplatin

the median overall survival (OS) was 10.30 months (95% CI, range 9.18–11.42 months).

GNAS1 T393C genotypes and clinicopathological parameters

The correlation of *GNAS1* T393C genotypes with various clinicopathological characteristics was shown in Table 1. Thirty-three (25.2%) patients displayed a CC genotype, 63 (47.4%) with a CT genotype and 35 (26.7%) with a TT genotype. All genotype distribution was compatible with the Hardy–Weinberg equilibrium. However, no association was detected between the *GNAS1* T393C genotypes and clinicopathological features, including gender, age, histology, smoking status, and stage.

GNAS1 T393C genotypes and chemotherapy sensitivity

Among the 131 patients who were treated with gemcitabine plus platinum chemotherapy, 83 patients received the combination of gemcitabine and cisplatin regimens, while 48 patients got gemcitabine and carboplatin treatment. Patients with the TT genotype were more sensitive to gemcitabine plus platinum compared with patients with CT or CC genotype ($P = 0.025$; Table 2). Thirty-two patients in the group with TT genotype (32/35, 91.43%) showed good response of treatment (PR + SD), while 49 patients in the CT group (49/63, 77.78%) and 25 patients (25/33, 75.76%) in the CC group had good response, indicating a substantial difference in chemotherapy response rate based on the *GNAS1* T393C genotype.

Table 2 Association between *GNAS1* T393C genotypes and tumor responses of patients with advanced NSCLC ($n = 131$)

	All	*GNAS1* T393C genotype			*P*
		CC	CT	TT	
N	131	33	63	35	
PR	40	6	16	18	
SD	66	19	33	14	
PD	25	8	14	3	0.025

PR partial response, *SD* stable disease, *PD* progressive disease

Clinical outcome by *GNAS1* T393C genotypes

Tumor-specific overall survival and progress-free survival dependent on T393C genotypes were analyzed by using Kaplan–Meier survival curves. Overall survival stratified by the T393C genotypes was shown in Fig. 1a. Log-rank test

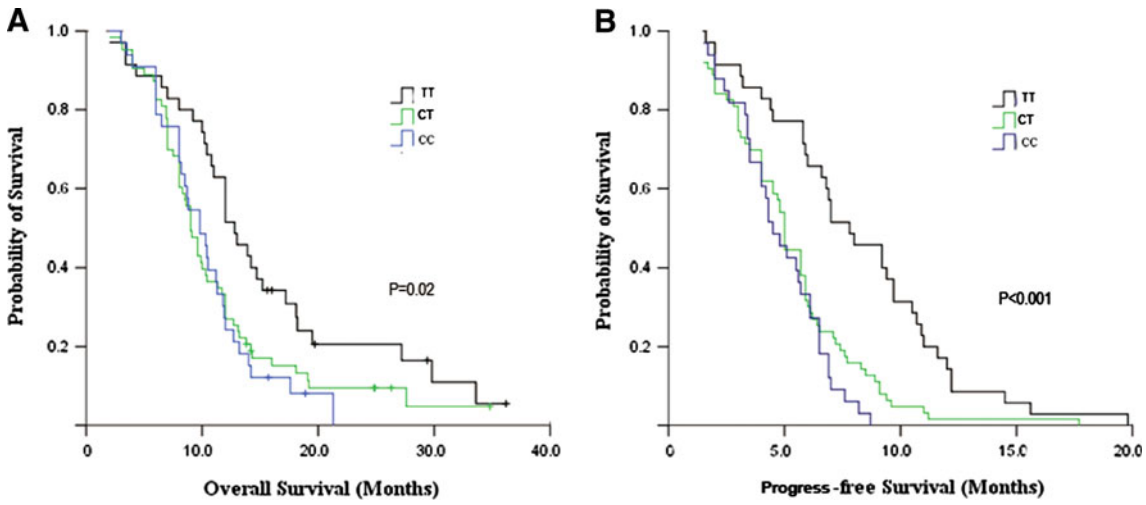

Fig. 1 Kaplan–Meier curves of overall survival **a** and progress-free survival **b** for advanced NSCLC patients with different *GNAS1* T393C genotype

showed that patients with TT genotype (median survival time: 12.80 months; 95% CI: 10.34–15.30) experienced a better survival than patients with CT genotype (median survival time: 9.80 months; 95% CI: 7.89–11.71) or TT genotype (median survival time: 9.00 months; 95% CI: 8.03–9.97) ($P = 0.02$). Progress-free survival dependent on T393C genotypes was shown in Fig. 1b. Again, the progress-free survival times for the T393C TT (7.80 months; 95% CI: 5.58–10.02) were longer than CT (5.00 months; 95% CI: 4.71–5.29) and CC (4.50 months; 95% CI: 3.49–5.51) ($P < 0.001$).

Multivariate Cox model analysis

Cox multivariate regression model was utilized to evaluate the prognostic value of T393C genotypes, sex, age, ECOG status, tumor stage, and chemotherapy in advanced NSCLC patients. *GNAS1* T393C genotype was found to be a strong disease-stage independent prognostic factor with marked increased risk of overall survival in CC genotype (HR 2.01, $P = 0.009$) and CT (HR 1.73, $P = 0.022$) genotype compared to TT patients (Table 3).

Table 3 Multivariate Cox regression analysis of factors possibly influencing overall survival in patients with advanced NSCLC ($n = 131$)

Variables	HR	95% CI	*P*
GNAS1			
TT	Ref		
CC	2.01	1.19–3.40	0.009
CT	1.73	1.08–2.75	0.022
Sex			
Male	Ref		
Female	1.065	0.56–2.02	0.848
Age			
<60	Ref		
≥60	1.07	0.70–1.53	0.864
ECOG			
≥2	Ref		
0–1	0.4	0.23–0.68	0.001
Stage			
IIIB	Ref		
IV	1.47	0.90–2.4	0.125
Histology			
Adeno	Ref		
SCC	1.15	0.76–1.75	0.515
Other	0.75	0.41–1.35	0.332
Chemotherapy			
Gem + Cisplatin	Ref		
Gem + Carbo	1.38	0.90–2.11	0.141

Discussion

Although platinum-based doublets (platinum plus another agent) have been commonly utilized in the treatment of lung cancer, there is wide variability in their observed effectiveness in individual patient [1, 9]. Identification of predictive markers for response to chemotherapy is most warranted, since a subgroup of the patients could not benefit from chemotherapy. In the current study, we found that inter-individual variations of the *GNAS1* T393C polymorphism may help predict chemotherapy efficiency and prognosis of patients with advanced NSCLC. Our results showed that GNAS1 T393 homozygous patients displayed a much longer survival than homozygous CC patients and heterozygous CT patients.

There is increasing evidence that induced cell apoptosis capacity is associated with improved survival with platinum-based chemotherapy [10, 11]. Polymorphic variants in apoptosis related genes could explain inter-individual difference in survival for platinum-treated NSCLC patients independently of ECOG status, the primary clinical prognostic factor. Previous studies have found that an increased concentration of the intracellular second messenger cyclic AMP, which is generated by activated Gαs, promotes apoptosis in several cell types. Furthermore, it has been reported that increased Gαs expression in patients carrying GNAS1 393T allele may enhance cellular apoptosis in cancer cells [8]. Taken together, understanding the correlation between the genotypes of apoptosis related genes and the efficacy of chemotherapy in NSCLC can further elucidate how certain polymorphism adversely or favorably affects chemotherapy outcomes [12, 13].

The relationship between GNAS1 T393C polymorphism and patient's prognosis may vary in different tumor types. In this study for NSCLC, together with other studies in bladder cancer [8], clear cell renal carcinoma [14], colorectal cancer [15], and gastric cancer [16], we showed significantly higher survival rate for TT genotype. However, in the invasive breast cancer [7] and intrahepatic cholangiocarcinoma [17], the CC genotype patients had more favorable clinical course. Further studies need to be done to find out the molecular and cellular mechanisms underlying the significance of the T393C genotypes in different tumor types and cellular drug responses.

It has been reported that PR and SD derived from the initial radiographic response after the first two courses of platinum-based chemotherapy showed similar PFS and OS for patients with advanced NSCLC [18]. In the present study, we found that the *GNAS1* T393C TT genotype showed much better chemotherapy reaction, making *GNAS1* T393C as an independent predictive biomarker for chemotherapy sensitivity and prognosis.

One of the most important strengths in this study is that all the patients were only received platinum-based chemotherapy, excluding the surgical resection, chemotherapy with multiple anticancer drugs, and radiotherapy affect. Such a homogeneously treated group of patients enable us to find out whether the genotypes of *GNAS1* T393C influence survival for advanced NSCLC primarily in the context of this particular regimen. However, we also acknowledge several limitations of this study. One limitation is that it is just a single-hospital retrospective research. Multicenter and prospective research is needed to further assess the prognostic value of GNAS1 SNP in the advanced NSCLC patients. Another limitation is that majority of the patients (106/131) are at stage IV. Thus, we could not provide sufficient statistical power to verify tumor stage is an independent prognosis factor in Cox's multivariate model analysis.

In summary, to our knowledge, this is the first study to identify SNP markers prognostic to targeted regimens in the GNAS1 gene for advanced NSCLC patients with gemcitabine plus platinum treatment. Hopefully, the *GNAS1* T393C SNP will improve the prediction of advanced NSCLC patient sensitivity to GP regimens. However, the prognostic value of the SNP marker reported need to be further validated in large cohorts. Furthermore, the regulatory pathway associated with potentially surrogate SNPs remains to be explored.

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Conflict of interest The authors declare that they have no competing interests.

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