TT genotype of GNAS1 T393C polymorphism predicts better outcome of advanced non-small cell lung cancer patients

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Abstract

AIM: To evaluate the potential prognostic value of GNAS1 T393C polymorphism in advanced non-small cell lung cancer.

METHODS: We extracted genomic DNA from the peripheral blood leukocytes of 94 patients with advanced non-small cell lung cancer. Quantitative real-time polymerase chain reaction was used to determine the genotyping of genomic DNA. Zhu F gave suggestions in writing the article; Song QB directed and coordinated the study; all authors were involved in organizing and refining the article.

RESULTS: Thirty-eight out of 94 (40%) patients displayed a TT genotype, 29 out of 94 (31%) a CT genotype and 27 out of 94 (29%) a CC genotype. The median survival of TT (25 mo) genotype carriers was longer than CT (12 mo) or CC (8 mo) genotype carriers. The favorable TT genotype predicted better overall survival (OS) (2-year OS: 48%; P = 0.01) compared with CT (2-year OS: 18%) or CC (2-year OS: 15%) genotype. However, dichotomization between C-genotypes (CC + CT) and T-genotypes (TT) revealed significantly lower survival rates (2-year OS: 16%; P = 0.01) for C allele carriers.

CONCLUSION: Our data provided strong evidence that the GNAS1 T393C genetic polymorphism influenced the prognosis in advanced non-small lung cancer with a worse outcome for C allele carriers.

Key words: GNAS1; Polymorphism; Advanced non-small cell lung cancer; Prognosis

INTRODUCTION

The incidence of lung cancer has increased substantially over the past ten years[1]. Non-small cell lung cancer (NSCLC) constitutes about 85% of all lung cancer cases[2] with only 16.6% being able to live 5 years or more.
after diagnosis\textsuperscript{[3]}. To date, the most feasible treatment for advanced NSCLC patients is the platinum-based combination chemotherapy and it turns out to be associated with better overall survival rates\textsuperscript{[4]}. Tumor-node-metastasis stage normally correlates with the clinical outcome of a large population of patients, but patients with similar clinical characteristics have different outcomes, which may be affected by their individual genes. The identification of patients with high-risk lung cancer could thus help to set up novel treatment strategies and could theoretically improve the outcome of anti-cancer therapy. Therefore, it is desirable to characterize more reliable and accurate molecular markers to identify more aggressive lung cancer phenotypes in order to individually tailor the therapy.

Actually, previous studies have implied that biomarkers could help define the subgroups of patients. However, there is no standard way to immunohistochemically detect these biomarkers, which prevents their application as prognostic factors. Nowadays, people choose to study single nucleotide polymorphisms (SNPs) as prognostic markers because these SNPs can be easily evaluated using patients’ blood samples, which can avoid issues such as the availability and the quality of materials. One typical example is the \textit{GNAS1} T393C polymorphism.

The \textit{GNAS1} gene has been mapped to chromosome 20q13 and contains 13 exons. The \textit{GNAS1} T393C polymorphism is located in exon 5, which encodes the \( \alpha \)-subunit of the stimulatory G protein, namely G\( \alpha \s \). Somatic mutations of \textit{GNAS1} have been reported to be involved in the etiology of McCune-Albright syndrome and sporadic, isolated endocrine tumors\textsuperscript{[5-7]}, suggesting that \textit{GNAS1} could participate in cancer initiation and progression. What’s more, previous studies have demonstrated that the T393C polymorphism was significantly correlated with the prognosis of patients with various cancers, such as breast carcinoma, squamous cell carcinoma of the larynx, bladder cancer, cholangiocarcinoma, colorectal cancer, clear cell renal carcinoma, and cancers of the oropharynx and hypopharynx\textsuperscript{[8-20]}.

In this study, we genotyped the \textit{T393C} SNP in a Han population to evaluate the effect of this polymorphism on lung cancer prognosis. Our purpose was to determine whether the common \textit{GNAS1} T393C polymorphism can be used as a predictive factor for survival in NSCLC patients.

**MATERIALS AND METHODS**

**Patients and clinical samples**

Two milliliters of peripheral blood samples were collected from patients diagnosed with advanced NSCLC pathologically before any antineoplastic therapy at Renmin Hospital of Wuhan University (China) between March 2010 and March 2012. Patients were chosen based on the following criteria: (1) histologically confirmed UICC (2009) stage \( \text{III B or IV NSCLC} \); (2) Eastern Cooperative Oncology Group performance status (PS) score of 2 or less; and (3) life expectancy of more than 3 mo. Patients were not included if they had received any anti-tumor therapy previously. All patients were asked to sign the informed consent before they were included in the database. The study cohort (94 patients; for clinicopathological data, Table 1) composed exclusively of patients with a meticulously complete follow-up record. This study was performed following the guidelines of the local research ethics committee.

**DNA extraction and genotyping**

Genomic DNA was isolated from whole blood samples using the QIAamp kit (Qiagen, Germany). \textit{T393C} SNP (dbSNP rs7121) was amplified by polymerase chain reaction (PCR) with the following primers: 5’-CAGGTCTGTTGCATTAGGGAGCATAT-3’ (forward) and 5’-TAATCCCTGCTATGCTACGGA-3’ (reverse). After denaturation at 95 °C, 50 cycles of DNA amplification was done using (NH\(_4\))\(_2\)SO\(_4\) containing buffer (Bioron, Germany) at 95 °C for 60 s, 60 °C for 30 s, and 70 °C for 60 s. The 807-bp PCR product was genotyped according to their sequences.

**Statistical analysis**

The software SPSS 17.0 was used for statistical analyses in this study. Descriptive statistics were applied to describe patients’ baseline characteristics. The correlation between \textit{T393C} genotypes and the clinical outcome was evaluated by Kaplan-Meier plots and the log-rank test. The survival time was calculated from the date of the primary diagnosis to the end of follow-up or date of death, whichever occurred first. The independent influence of \textit{T393C} SNP and other covariates on survival rates was assessed in multivariate analysis using the Cox regression hazard model. \( P \) values < 0.05 were considered statistically significant. The compatibility with the Hardy-Weinberg equilibrium was calculated with HWE program (http:// linkage.rockefeller.edu/ott/linkutil.htm).

**RESULTS**

**Analysis of \textit{GNAS1} T393C genotypes and associated clinicopathological features**

The clinicopathological characteristics of patients with genotype distribution are shown in Table 1. There were 94 advanced NSCLC patients participating in this study, including 23 women and 71 men. The average age of participants was 58.6 years (range, 31 to 80 years).

Among 94 patients, 38 (40%) displayed a TT genotype, 29 (31%) with a CT genotype and 27 (29%) with a CC genotype. In the entire patient group, the frequency of the C allele (\( \text{CC} \)) was 0.55. The distribution was compatible with the Hardy-Weinberg equilibrium. There was no significant correlation between the \textit{GNAS1} \textit{T393C} genotypes and clinicopathological parameters, such as age (\( P = 0.48 \)), gender (\( P = 0.42 \)), PS (\( P = 0.30 \)), smoking status (\( P = 0.44 \)) or pathology (\( P = 0.59 \)) (Table 2). Further analysis showed that there was no significant correlation of overall survival (OS) with age (\( P = 0.135 \)), gender (\( P = 0.0580 \)), PS (\( P = 0.658 \)), smoking (\( P = 0.473 \)), pathology (\( P = 0.559 \)), or treatment mode (\( P = 0.116 \)).
**Table 1** Clinicopathological characteristics of 94 patients with non-small cell lung cancer

| Subgroup                  | All (n = 94) | TT (n = 38; 40%) | TC (n = 29; 31%) | CC (n = 27; 29%) | P     |
|---------------------------|--------------|-----------------|-----------------|-----------------|-------|
| Gender                    |              |                 |                 |                 |       |
| Male                      | 71           | 31 (43.6)       | 22 (39.6)       | 18 (25.5)       | 0.42  |
| Female                    | 23           | 7 (30.4)        | 7 (30.4)        | 9 (39.2)        |       |
| Age                       |              |                 |                 |                 |       |
| > 60 yr                   | 51           | 22 (43.1)       | 13 (25.5)       | 16 (31.4)       | 0.48  |
| < 60 yr                   | 43           | 16 (37.2)       | 16 (37.2)       | 11 (25.6)       |       |
| Performance status        |              |                 |                 |                 |       |
| > 2                       | 25           | 13 (52.0)       | 5 (20.0)        | 7 (28.0)        | 0.30  |
| < 2                       | 69           | 25 (36.2)       | 24 (34.8)       | 20 (29.0)       |       |
| Smoking                   |              |                 |                 |                 |       |
| Yes                       | 23           | 12 (52.2)       | 5 (26.3)        | 5 (21.7)        | 0.44  |
| No                        | 71           | 26 (36.6)       | 23 (32.4)       | 22 (31.0)       |       |
| Pathology                 |              |                 |                 |                 |       |
| Adenocarcinoma            | 48           | 19 (39.6)       | 17 (35.4)       | 12 (25.0)       | 0.59  |
| Squamous cell carcinoma   | 46           | 19 (41.3)       | 12 (26.1)       | 15 (32.6)       |       |

Table 2  Association between *GNAS1 T393C* single nucleotide polymorphism and clinical parameters

**GNAS1 T393C TT genotype predicts favorable survival**

The median survival of carriers of TT, CT and CC genotypes was 25, 12, and 8 mo, respectively. We analyzed the relationship between overall survival rate and *T393C* genotypes using Kaplan-Meier survival curves. Our data showed that the favorable TT genotype was significantly associated with better OS (2-year OS: 48%; *P* = 0.01) when compared with the other genotypes. For example, the 2-year OS for CT genotype was 18% and 15% for CC genotype (Figure 1). By applying the multivariate Cox proportional hazards model, we found that *GNAS1 T393C* polymorphism was independently associated with OS after adjusting the clinicopathological factors (*P* < 0.05). However, the dichotomization between C-genotypes (CC + CT) and T-genotypes (TT) indicated significant lower survival rates for C-allele carriers (*P* = 0.01), which had a 2-year OS rate of 16% (Figure 2).

**DISCUSSION**

Lung cancer is the major cause of cancer death in the world and there is an urgent need to accurately and individually treat patients with lung cancer. Although clinicopathological parameters such as UICC stage may serve as prognostic markers in lung cancer, it is still desirable to develop more reliable and accurate biomarkers to more precisely predict the clinical outcome of individual patients. Most prognostic biomarkers are developed according to the features of the tumor tissue itself. The *GNAS1* gene encodes the Gαs subunit of G protein and it has been shown that the *GNAS1 T393C* polymorphism correlates with lung cancer [20]. Hence, we investigated whether *GNAS1 T393C* polymorphism can be used to predict the clinical outcome in patients with NSCLC. Our study clearly indicated that the homozygous TT genotype patients had a much higher survival rate than patients with either homozygous CC or heterozygous CT genotype. If we could identify patients with poor clinical outcome, we might develop novel treatment strategies accordingly at the initial stage of management, which could lead to improved individual therapy strategies with higher survival rates. Meanwhile, our results also indicated the potential role of the *GNAS1 T393C* polymorphism as a possible general genetic marker for tumor progression and survival since T-allele carriers demonstrated better clinical outcome than C-allele carriers (TC and CC genotypes). However, it should be noted that the connection between *GNAS1 T393C* polymorphism and survival was
compared with CT or CC genotype. For example, in advanced squamous cell carcinoma of the larynx, the five
different in different types of tumors. For some tumors, TT genotype was significantly correlated with better OS
year survival rate for TT genotype patients was 76%, 49% for TC genotype, and 43.5% for CC genotype\cite{10}. Also, it had been reported that the five-year survival rate of sporadic colorectal cancer patients with a TT genotype (87.8%) was much higher than that of patients with a TC (71.0%) or CC genotype (50.0%)\cite{8}. On the other hand, in intrahepatic cholangio-carcinoma\cite{9}, esophageal cancer\cite{5}, and breast cancer\cite{6}, the patients with a CC genotype had a more favorable clinical outcome (Table 3). Thus, it was conceivable that the GNAS1 T393C polymorphism in various tumor types had different biological effects. In order to understand the significance of the T393C genotypes in different tumor types, further more studies are needed to clarify the molecular mechanisms.

In vitro studies demonstrated that increased Gsα expression promotes apoptosis\cite{21}. Therefore, it is highly likely that increased Gsα expression and the subsequently increased apoptosis could be associated with better survival rate in patients with a GNAS1 TT genotype. In vitro experiments also suggest that the product of Gsα, cyclic AMP, could play a crucial role in the proapoptotic process. It has been reported that increasing the intracellular concentration of cyclic AMP leads to enhanced apoptosis in several cell lines including lymphoma cells\cite{22}, leukemic\cite{23} and ovarian cancer cells\cite{24}. Gsα was also found to be differentially expressed between various GNAS1 T393C genotypes. Previous studies have suggested that Gsα transcription level is increased in individuals with a GNAS1 393 TT genotype\cite{8}. Intriguingly, the mRNA stability has been shown to be determined by the coding region of some genes\cite{25-27}. Using the MFOLD (the software for the prediction of the secondary structure of single stranded nucleic acids), Alakus et al\cite{8} have reported that the substitution of T393 to C affects the structure of mRNA, most likely the mRNA folding.

Several biomarkers have been used as predictive and prognostic markers for NSCLC patients. A prognostic biomarker is a molecule that can be used to indicate the patient survival independent of the treatment received. In other words, it is an indicator of the innate tumor aggressiveness. For example, KRAS mutations can serve as a good prognostic biomarker indicating the poor survival for NSCLC patients when compared with the patients without KRAS mutations, independent of therapy. Xie et al\cite{28} has reported that the GNAS1 T393C polymorphism can somehow predict the chemotherapy sensitivity and overall survival rate in advanced NSCLC patients treated with gemcitabine and platinum\cite{29}. Here, our data clearly indicate that the GNAS1 T393C TT genotype was prognostic of better overall survival for NSCLC patients, independent of therapy. Nevertheless, it should be emphasized that in this study, we only investigated a small population of patients. Although our study indicated that genetic host factors play a role in tumor progression, which was consistent with the previously published data\cite{29}, further independent studies of large cohorts are necessary to confirm the reliability of our findings. Furthermore, the molecular mechanisms underlying the significance of the GNAS1 T393C genotype associated with potentially surrogate SNPs remain to be explored.

COMMENTS

Background

Lung cancer is major cause of cancer death around the world. Although some clinicopathological parameters like UICC stage may be used as prognostic biomarkers in lung cancer, other reliable markers that can help precisely predict the clinical outcome of individual patients are still desirable. Most prognostic biomarkers are based on features of the tumor tissue itself.

Research frontiers

Characterization of single nucleotide polymorphisms (SNPs) as a prognostic biomarker in cancer has become the hotspot of recent research. The T393C polymorphism of the GNAS1 gene is one such polymorphism.

Innovations and breakthroughs

Several molecular markers have been used as predictive and prognostic markers for non-small cell lung cancer (NSCLC). A prognostic biomarker is a biomolecule that can be used to indicate the patient survival independent of the treatment received. It can also indicate for the innate tumor aggressiveness. For example, the KRAS mutations are prognostic of poor survival for NSCLC patients when compared to the absence of KRAS mutations, independent of therapy. Xie et al reported that the GNAS1 T393C polymorphism can be used to predict the chemotherapy sensitivity as well as the survival rates in advanced NSCLC patients treated with gemcitabine and platinum. Here, the data clearly indicate that the GNAS1 T393C TT genotype was prognostic of better survival rates for NSCLC patients, independent of therapy.

Applications

The identification of patients with high-risk lung cancer could help develop novel and individual treatment strategies and could improve the clinical outcome. This data clearly indicate that genetic polymorphism in the GNAS1 T393C influenced survival in advanced non-small lung cancer with a worse clinical outcome for C allele patients.

Terminology

SNPs refer to a DNA sequence variation occurring commonly within a population (e.g., 1%) in which a single nucleotide -A, T, C or G - in the genome (or other shared sequence) differs between members of a biological species or other shared sequence).

Peer review

The manuscript is comprehensive and important.

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