**ORIGINAL ARTICLE**

**P73 G4C14-to-A4T14 polymorphism is associated with survival in advanced non-small cell lung cancer patients**

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**Keywords**
Chemotherapy; non-small cell lung cancer; p73; platinum; polymorphism.

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Received: 18 July 2016;
Accepted: 11 September 2016.

doi: 10.1111/1759-7714.12397

**Thoracic Cancer** 8 (2017) 63–72

**Abstract**

**Background:** p73, a structural and functional homolog of p53, plays an important role in modulating cell cycle arrest. This study investigated the association between p73 G4C14-to-A4T14 polymorphism and survival outcomes in a Chinese population of advanced non-small cell lung cancer (NSCLC) patients treated with platinum agents.

**Methods:** The p73 G4C14-to-A4T14 polymorphism was genotyped using DNA from blood samples of advanced NSCLC patients (642 in the discovery set and 330 in the replication set). The relationship of the p73 G4C14-to-A4T14 polymorphism with clinical outcomes was analyzed.

**Results:** Compared with the GC/GC genotype, the genotypes containing AT allele (GC/AT + AT/AT genotypes) were associated with significantly prolonged overall survival (P = 0.040) in the discovery set and after pooling results from the replication set. Stratification analysis revealed that the association was more pronounced in subjects who were older (P = 0.001), male (P = 0.007), smokers (P = 0.006), had a low Eastern Cooperative Oncology Group performance status (P = 0.001), in tumor node metastasis stage IV (P = 0.008), and with adenocarcinoma (P = 0.002). The objective response rates of patients with GC/AT + AT/AT genotypes were statistically higher than those with the GC/GC genotype (P = 0.047).

**Conclusion:** Our findings suggest that the p73 G4C14-to-A4T14 polymorphism may be related to survival outcome in advanced NSCLC patients.

**Introduction**

Non-small-cell lung cancer (NSCLC), accounts for approximately 85% of primary lung cancers and remains the leading cause of cancer-related death worldwide. Despite the encouraging improvement of treatment methods over the last decades, the five-year survival rate is still low, which is mainly attributed to the large proportion of advanced cases at the time of diagnosis. Combination therapy based on platinum agents has been the most common form of treatment for advanced NSCLC, regardless of various therapy responses between patients. However, the major problem is the optimization of treatment options, which could help clinicians determine which patients will benefit from which therapy.

The p73 gene, a structural and functional homolog of the p53 gene, plays a crucial role in the presence of DNA damage induced by platinum-based chemotherapy. The p73 G4C14-to-A4T14 polymorphism consists of two single nucleotide polymorphisms (SNPs, rs2273953 and rs1801173), which are in complete linkage disequilibrium,
located at positions 4 (G→A) and 14 (C→T) in the 5′ untranslated region (UTR) of exon 2, just upstream of the initiating AUG of the p73 gene. It has been shown that the GC to AT change may form a stem-loop structure and possibly affect the translation efficiency of p73. An increasing number of studies have investigated the relationship between the p73 G4C14-to-A4T14 polymorphism and the susceptibility of various cancers, including lung, breast, esophageal, prostate, cervical, and gastric carcinoma in different ethnic populations. In addition, evidence has also indicated that the expression level of the p73 gene is a non-ignorable factor of chemosensitivity in human tumors. However, in spite of the well-known impact of the p73 G4C14-to-A4T14 polymorphism on cancer development, its potential role in chemotherapeutic response and prognosis of NSCLC has not been fully investigated. To further test the association, we performed a two-stage association analysis for this validated polymorphism by conducting a discovery cohort with 642 advanced NSCLC patients who received platinum-based chemotherapy followed by further replication in an independent replication cohort with 330 patients in a Chinese population.

Methods

Study population and follow-up

The discovery set included 642 cases with confirmed late-stage (III–IV) NSCLC who had received platinum-based chemotherapy between March 2005 and January 2010 on the oncological departments of Shanghai Zhongshan Hospital, Shanghai Chest Hospital, and Shanghai Changzheng Hospital. Positive hits from the discovery set were validated in patients with advanced NSCLC from an independent replication cohort. This dataset included 330 advanced NSCLC cases from Shanghai Pulmonary Hospital between June 2010 and May 2013. Blood samples from all subjects were collected at the time of diagnosis, prior to chemotherapy treatment. All subjects provided written informed consent and the medical ethics committee of each participating institution approved the study.

Follow-up was performed every three months from the time of enrollment until death or the last follow-up. Data of all cases were collected retrospectively from the medical records and databases of each hospital. The definition of non-smokers used was described in a previous study. Overall survival (OS) was defined as the period from receipt of chemotherapy to the time of death or last follow-up. Progression-free survival (PFS) was defined as the duration from the first treatment to the date of disease progression, death or last follow-up. Therapeutic response was assessed after the first two or three cycles and determined by Response Evaluation Criteria in Solid Tumors version 1.1.

The disease control rate (DCR) included complete response (CR), partial response (PR) and stable disease (SD). The objective response rate (ORR) consisted of complete response (CR) and partial response (PR).

Chemotherapy regimens

All participants received first-line platinum-based chemotherapy; the detailed chemotherapeutic regimens have previously been described.

Genotype analysis

Blood samples were collected from each participant and genomic DNA was extracted using the Human Whole Blood Genomic DNA Extraction Kit (Qiagen, Valencia, CA, USA). We analyzed samples for the p73 G4C14-to-A4T14 polymorphism using the TaqMan Pre-Designed SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA) following the manufacturer’s instructions. The PCR primers used for amplifying p73 G4C14-A4T14 were as follows: 5′-CAGGAGGACAGACGAGTT-3′ (forward) and 5′-TGATGAGGTTGGCTAAGGCTA-3′ (reverse). Approximately 15% of the samples were randomly selected for repeat genotyping by a different investigator, and the results were entirely concordant.

Statistical analysis

The distribution of selected variables and p73 genotype frequencies between the discovery and replication sets were evaluated using the χ² test. Hardy–Weinberg equilibrium was tested by a goodness-of-fit χ² test to compare the observed genotype frequencies. Survival curves were computed according to Kaplan–Meier curves. Univariate analysis was conducted using Cox’s proportional hazard model to validate the significant variables related to survival. Multivariate analysis was then performed using variables with a univariate P < 0.1. For chemotherapeutic response, unconditional multivariate logistic regression analysis was performed to estimate odds ratios (ORs), along with the corresponding 95% confidence intervals (CIs) for p73 genotypes. All statistical analyses were accomplished using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) and P values <0.05 were considered statistically significant.

Results

Patient characteristics

The demographic and clinical characteristics of the patients in the two study cohorts are presented in Table 1. In the discovery set, 456 (71.0%) patients were male and 382 (59.5%) were smokers. All patients had advanced inoperable NSCLC,
with 40.8% in stage III and 59.2% in stage IV. The median OS and PFS of all patients were 19.27 (95% CI: 17.64–20.89) and 10.07 months (95% CI: 8.61–11.53), respectively. Patients with tumor node metastasis (TNM) stage IV and infrequent histological subtypes had significantly worse OS (\(P = 0.012\) and \(P = 0.027\), respectively) compared with patients with TNM stage III and adenocarcinoma (Fig 1a,b).

In addition, Eastern Cooperative Oncology Group (ECOG) performance status was related to PFS and patients with higher scores showed a higher risk of recurrence or metastasis (\(P = 0.006\); Fig 1c), whereas other clinical factors were not independent prognostic factors (Table 2).

Among the 330 cases in the replication set, 234 (70.9%) were men and 186 (56.4%) were smokers, with 36.1% in stage III and 63.9% in stage IV disease. TNM stage and tumor histology also showed significant associations with OS, similar to the discovery set (Table 3). There were no statistically significant differences in variables between the discovery and replication sets (Table 1).

### Survival analysis

#### Discovery set

All genotype frequencies for \(p73\) G4C14-to-A4T14 were in Hardy–Weinberg equilibrium (\(P > 0.05\)). There was no significant difference in the genotype distributions of \(p73\) G4C14-to-A4T14 according to clinical factors (Table 3). We classified this polymorphism by models including genotypic, dominant, recessive, and additive. The results demonstrated that individuals with the AT/AT genotype have prolonged OS compared with GC/GC carriers (adjusted hazard ratio [aHR] \(= 0.65\), \(P = 0.035\)), whereas heterozygotes showed no significance after adjusting for selected variables. In addition, patients carrying the AT allele (GC/AT or AT/AT) had significantly increased OS in the dominant model (for GC/AT + AT/AT genotype HR 0.82; \(P = 0.040\)). Kaplan–Meier curves also indicated these results (log-rank test for the genotypic model \(P = 0.019\), for the dominant model \(P = 0.021\); Fig 1d,e; Table 2). However, none of genotypes showed a significant relationship with PFS (data not shown).

#### Replication set and pooled analysis

To validate the association of \(p73\) G4C14-to-A4T14 with OS, another independent replication set with 330 advanced NSCLC cases was performed. In this second group, the \(p73\) G4C14-to-A4T14 genotypes showed a similar trend of relationship with survival. We concluded that carriers of the AT/AT genotype were significantly associated with

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**Table 1** Basic patient characteristics

| Variables                          | Discovery set (N, %) | Replication set (N, %) | \(\chi^2\) | \(P^*\) |
|------------------------------------|----------------------|------------------------|------------|--------|
| Age (years)                        |                      |                        |            |        |
| <58                                | 333 (51.9)           | 171 (51.8)             | 0.001      | 0.988  |
| ≥58                                | 309 (48.1)           | 159 (48.2)             |            |        |
| Gender                             |                      |                        |            |        |
| Male                               | 456 (71.0)           | 234 (70.9)             | 0.001      | 0.969  |
| Female                             | 186 (29.0)           | 96 (29.1)              |            |        |
| Smoking history                    |                      |                        |            |        |
| Non-smokers                        | 260 (40.5)           | 144 (43.6)             | 0.884      | 0.347  |
| Smokers                            | 382 (59.5)           | 186 (56.4)             |            |        |
| ECOG PS                            |                      |                        |            |        |
| 0–1                                | 593 (92.4)           | 297 (90.0)             | 1.582      | 0.209  |
| 2                                  | 49 (7.6)             | 33 (10.0)              |            |        |
| Chemotherapy                       |                      |                        |            |        |
| NP/NC                              | 236 (36.8)           | 115 (34.8)             | 1.126      | 0.771  |
| GP/GC                              | 174 (27.1)           | 95 (28.8)              |            |        |
| TP/TC                              | 192 (29.9)           | 95 (28.8)              |            |        |
| DP/DC                              | 40 (6.2)             | 25 (7.6)               |            |        |
| TNM stage                          |                      |                        |            |        |
| III                                | 262 (40.8)           | 119 (36.1)             | 2.063      | 0.151  |
| IV                                 | 380 (59.2)           | 211 (63.9)             |            |        |
| Tumor histology                    |                      |                        |            |        |
| Adeno                              | 398 (62.0)           | 213 (64.5)             | 2.057      | 0.358  |
| SQC                                | 147 (22.9)           | 66 (20.0)              |            |        |
| Others                             | 97 (15.1)            | 51 (15.5)              |            |        |

*\(P\)-values derived from \(\chi^2\) test. Adeno, adenocarcinoma; DP/DC, carboplatin or cisplatin plus docetaxel; ECOG PS, Eastern Cooperative Oncology Group performance status; GP/GC, carboplatin or cisplatin plus gemcitabine; NP/NC, carboplatin or cisplatin plus vinorelbine; SQC, squamous cell carcinoma; TNM, tumor node metastasis; TP/TC, carboplatin or cisplatin plus paclitaxel.
increased OS compared with the GC/GC genotype (aHR = 0.64, \( P = 0.040 \)). The dominant (\( P = 0.014 \)) and additive models (\( P = 0.015 \)) of \( p73 \) G4C14-to-A4T14 also showed a statistically significant association (Table 4).

Further pooled analysis of the two cohorts verified the previously observed association of the \( p73 \) G4C14-to-A4T14 polymorphism with OS. Kaplan–Meier curves and log-rank tests showed that both the AT/AT variant homozygotes and the GC/AT heterozygotes were significantly correlated with prolonged OS compared with the GC/GC homozygotes in the 972 NSCLC patients (\( P = 0.001 \); Fig 1f). Univariate Cox regression analysis also confirmed this association (for GC/AT + AT/AT genotype HR = 0.80, \( P = 0.003 \); for AT/AT genotype HR = 0.69, \( P = 0.032 \); for additive model HR = 0.82, \( P = 0.001 \)) after adjusting for clinical variables (Table 3). Furthermore, we included age (\( \geq 58 \) vs. <58), TNM stage (TNM IV vs. TNM III), tumor histology (adenocarcinoma, squamous, or others), and the \( p73 \) G4C14-to-A4T14 dominant and recessive models in multivariate Cox regression analysis. The results suggested that TNM stage (\( P = 0.004 \)), tumor histology (\( P = 0.022 \)), and dominant model (\( P = 0.017 \)) were independent predictive factors for OS (Table 5).

**Stratification analysis**

To better understand the potential impact of the \( p73 \) G4C14-to-A4T14 polymorphism on survival in NSCLC patients, we performed subgroup analysis stratified by confounding variables in the pooled populations. Because the AT/AT genotype was relatively infrequent, we combined it with the GC/AT genotype for further examination. As shown in Table 6, the favorable effect of the \( p73 \) combined genotypes (GC/AT + AT/AT) was more evident in patients...
who were older (≥58 years) at diagnosis (P = 0.001), male (P = 0.007), smokers (P = 0.006), had a low ECOG performance status (0–1; P = 0.001), in TNM stage IV (P = 0.008), and with adenocarcinoma (P = 0.002). However, we did not find any interaction between the p73 variant genotypes and chemotherapeutic regimens for overall survival in NSCLC patients.

**P73 G4C14-to-A4T14 and chemotherapy efficacy**

The chemotherapeutic response of patients was assessed in the pooled populations. Disease control was noted in 761 (78.3%) patients, and objective response was achieved in 163 (16.8%). A marginally significant association with ORR other DCR for p73 G4C14-to-A4T14 was manifested by multivariate logistic regression analysis in the 972 NSCLC cases (for GC/AT + AT/AT genotype OR, 0.69; P = 0.047; Table 7).

**Discussion**

The principal finding of the present study is that the p73 G4C14-to-A4T14 polymorphism may be related to survival outcomes in advanced NSCLC patients who receive platinum-based chemotherapy. Given the role of p73 as an important regulator of cell cycle and DNA repair, it is...
showed that individual variability of drug response. Two landmark articles suggested that this validated polymorphism of p73 gene variant relevant to DNA damage may contribute to fully understanding or predicting the effect of chemotherapy. Recently, an increasing number of studies have suggested that this validated polymorphism of p73 not only influences the development, but also has an impact on the progression and prognosis of various cancers. For example, Carastro et al. demonstrated that p73 G4C14-to-A4T14 has a significant inverse relationship with aggressiveness and a marginal association with overall survival in prostate cancer.²² Pfeifer et al. reported that colorectal cancer patients with the AT allele had a better prognosis than those with the GC/GC genotype.²³ Lee et al. concluded that the p73 GC/AT genotype is associated with increased risk and survival of colorectal cancer in a Korean population.²⁴ Liu et al. demonstrated the combined effect of genetic polymorphisms in the p53, p73, and MDM2 genes on NSCLC survival.²⁵ However, the fairly small sample size and marginal significance mean that these results should not be interpreted as definitive. The current understanding of how p73 polymorphisms impact chemotherapy response is limited.

### Table 3: Distribution of p73 G4C14-to-A4T14 genotypes according to clinical factors

| Variables          | Discovery set, n (%) | Replication set, n (%) |
|--------------------|----------------------|------------------------|
|                    | GC/GC (n = 387)   | GC/AT (n = 212)   | AT/AT (n = 43) | χ² | P* |
| Age (years)        |                     |                       |               |    |    |
| <58                | 202 (60.7)         | 112 (33.6)          | 19 (5.7)      | 1.112 | 0.574 |
| ≥58                | 185 (59.9)         | 100 (32.4)          | 24 (7.8)      | 1.011 | 0.545 |
| Gender             |                     |                       |               |    |    |
| Male               | 280 (61.4)         | 148 (32.5)          | 28 (6.1)      | 1.112 | 0.545 |
| Female             | 107 (57.5)         | 64 (34.4)           | 15 (8.1)      | 1.011 | 0.545 |
| Smoking history    |                     |                       |               |    |    |
| Non-smokers        | 150 (57.7)         | 90 (34.6)           | 20 (7.7)      | 1.467 | 0.480 |
| Smokers            | 237 (62.0)         | 122 (31.9)          | 23 (6.0)      | 1.011 | 0.545 |
| ECOG PS            |                     |                       |               |    |    |
| 0–1                | 355 (59.9)         | 196 (33.1)          | 42 (7.1)      | 1.942 | 0.379 |
| 2                  | 32 (65.3)          | 16 (32.7)           | 1 (2.0)       | 1.011 | 0.545 |
| Chemotherapy       |                     |                       |               |    |    |
| NP/NC              | 137 (58.1)         | 83 (35.2)           | 16 (6.8)      | 6.962 | 0.324 |
| GP/GC              | 101 (58.0)         | 59 (33.9)           | 14 (8.0)      | 5.467 | 0.147 |
| TP/TC              | 127 (66.1)         | 57 (29.7)           | 8 (4.2)       | 5.030 | 0.135 |
| DP/DC              | 22 (55.0)          | 13 (32.5)           | 5 (12.5)      | 1.011 | 0.545 |
| TNM stage          |                     |                       |               |    |    |
| III                | 161 (61.5)         | 78 (29.8)           | 23 (8.8)      | 4.379 | 0.112 |
| IV                 | 226 (59.5)         | 134 (35.3)          | 20 (5.3)      | 2.682 | 0.102 |
| Tumor histology    |                     |                       |               |    |    |
| Adeno              | 236 (59.3)         | 133 (33.4)          | 29 (7.3)      | 1.307 | 0.860 |
| SQC                | 93 (63.3)          | 45 (30.6)           | 9 (6.1)       | 0.101 | 0.920 |
| Others             | 58 (59.8)          | 34 (35.1)           | 5 (5.2)       | 0.710 | 0.699 |

*P values derived from χ² test. Adeno, adenocarcinoma; ECOG PS, Eastern Cooperative Oncology Group performance status; GP/GC, carboplatin or cisplatin plus gemcitabine; m, months; NP/NC, carboplatin or cisplatin plus vinorelbine; SQC, squamous cell carcinoma; TNM, tumor node metastasis; TP/TC, carboplatin or cisplatin plus paclitaxel.*

biologically plausible that this well known SNP may potentially modulate the chemotherapy efficacy of DNA-damaging anti-cancer drugs, including platinum agents.

DNA damage in the G1 phase induced by platinum agents leads to the activation of cell cycle checkpoints, such as the p53 tumor suppressor, resulting in either G1 arrest or programmed cell death.¹⁴ Like p53, p73 has also been shown to respond to DNA damage, causing induction of cell cycle arrest or apoptosis.¹⁵ Because genetic polymorphisms in DNA repair genes, including the p53 Arg72Pro polymorphism, they potentially influence the activity of chemotherapeutic agents. We hypothesized that p73 genetic polymorphisms could also contribute to the individual variability of drug response. Two landmark articles showed that p73 is an important determinant of chemosensitivity in humans, and its function is highly integrated with that of p53.¹⁶,¹⁷ Several other articles have confirmed the importance of p73 expression in the prediction of tumor chemosensitivity and cancer prognosis by studying different tumor types.¹⁸–¹⁰ The regulatory mechanisms are primarily linked to posttranslational modifications and protein-protein interactions involving both signaling molecules and transcription factors. Indeed, mechanical behavior may be complex and variable, as it is dependent on the specific drugs and tissues involved. Thus, research on the genetic variants of the p73 gene relevant to DNA damage may contribute to fully understanding or predicting the effect of chemotherapy.

Recently, an increasing number of studies have suggested that this validated polymorphism of p73 not only influences the development, but also has an impact on the progression and prognosis of various cancers. For example, Carastro et al. demonstrated that p73 G4C14-to-A4T14 has a significant inverse relationship with aggressiveness and a marginal association with overall survival in prostate cancer.²² Pfeifer et al. reported that colorectal cancer patients with the AT allele had a better prognosis than those with the GC/GC genotype.²³ Lee et al. concluded that the p73 GC/AT genotype is associated with increased risk and survival of colorectal cancer in a Korean population.²⁴ Liu et al. demonstrated the combined effect of genetic polymorphisms in the p53, p73, and MDM2 genes on NSCLC survival.²⁵ However, the fairly small sample size and marginal significance mean that these results should

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| Variables | Replication set | Pooled populations |
|-----------|----------------|--------------------|
| N (%)     | OS (95% CI) (m) | Ref.               | N (%)     | OS (95% CI) (m) | Ref.               |
| Age (years) |                |                    |           |                |                    |
| <58       | 21.87 (17.35–26.38) | 0.038 Ref. | 21.30 (19.25–23.35) | 0.014 Reference |
| ≥58       | 17.57 (14.67–20.47) | 1.33 (1.02–1.741) | 1.17 (15.30–19.03) | 1.20 (1.03–1.40)  |
| Gender    |                |                    |           |                |                    |
| Male      | 19.93 (15.31–20.55) | 0.063 Ref. | 18.27 (16.64–19.89) | 0.057 Reference |
| Female    | 22.50 (16.47–28.53) | 0.85 (0.60–1.18)  | 21.43 (17.95–24.92) | 0.87 (0.69–1.10)  |
| Smoking history |  |                    |           |                |                    |
| Non-smokers | 22.50 (17.87–27.13) | 0.125 Ref. | 21.03 (18.69–23.38) | 0.025 Reference |
| Smokers   | 17.57 (15.13–20.00) | 1.18 (0.88–1.57)  | 17.93 (16.23–19.64) | 1.05 (0.85–1.29)  |
| ECOG PS   |                |                    |           |                |                    |
| 0–1       | 19.40 (16.21–22.59) | 0.482 Ref. | 19.40 (17.93–20.87) | 0.081 Reference |
| ≥2        | 18.00 (17.13–28.87) | 1.26 (0.80–1.89)  | 17.90 (11.14–24.66) | 1.22 (0.94–1.59)  |
| Chemotherapy |            |                    |           |                |                    |
| NPNC      | 17.87 (13.39–22.34) | 0.687 Ref. | 18.63 (16.14–21.13) | 0.399 Reference |
| GP/GC     | 19.07 (13.67–24.46) | 1.16 (0.84–1.60)  | 19.07 (16.37–21.76) | 0.95 (0.79–1.14)  |
| TP/TC     | 19.83 (15.48–24.19) | 1.07 (0.78–1.48)  | 19.27 (17.25–21.29) | 1.01 (0.85–1.22)  |
| DP/DC     | 20.90 (11.13–30.69) | 0.77 (0.42–1.40)  | 22.00 (18.01–25.39) | 0.81 (0.59–1.12)  |
| TNM stage |                |                    |           |                |                    |
| III       | 21.43 (17.43–25.44) | 0.018 Ref. | 21.03 (19.06–23.01) | 0.012 Reference |
| IV        | 17.87 (14.72–21.02) | 1.39 (1.06–1.834) | 17.87 (16.04–19.70) | 1.30 (1.12–1.52)  |
| Tumor histology |          |                    |           |                |                    |
| Adeno     | 20.67 (17.13–24.20) | 0.081 Ref. | 20.57 (18.71–22.43) | 0.007 Reference |
| SQC       | 17.57 (12.86–22.27) | 1.27 (0.89–1.68)  | 16.63 (13.12–20.15) | 1.16 (0.96–1.42)  |
| Others    | 18.00 (14.48–21.53) | 1.58 (1.08–2.32)  | 16.40 (13.11–19.69) | 1.37 (1.10–1.70)  |
| P73 G4C14-to-A4T14 | |                    |           |                |                    |
| GC/GC     | 16.37 (14.23–18.50) | 0.035 Ref. | 17.00 (15.36–18.64) | 0.001 Reference |
| GC/AT     | 24.03 (18.76–29.31) | 0.74 (0.55–0.99)  | 21.87 (19.08–24.66) | 0.93 (0.71–0.97)  |
| AT/AT     | 27.43 (15.97–38.90) | 0.64 (0.38–1.06)  | 29.80 (22.32–37.28) | 0.65 (0.47–0.89)  |
| Dominant  |                |                    |           |                |                    |
| GC/GC + AT/AT | 25.13 (20.66–29.61) | 0.015 Ref. | 22.63 (20.04–25.23) | 0.001 Reference |
| GC/AT     | 26.79 (24.37–28.80) | 0.72 (0.55–0.94)  | 29.80 (22.32–37.28) | 0.014 0.69 (0.51–0.95) |
| GC/GC     | 16.37 (14.23–18.50) | 0.040 Ref. | 19.83 (17.53–20.34) | 0.032 0.81 (0.68–0.93) |
| Additive  |                |                    |           |                |                    |
| NA        | 32.83 (22.11–43.56) | 0.083 Ref. | 69 (7.1) | 29.80 (22.32–37.28) | 0.014 0.69 (0.51–0.95) |
| NA        | 18.93 (16.26–21.61) | 0.77 (0.63–0.95)  | NA       | 18.93 (16.26–21.61) | 0.032 0.81 (0.68–0.93) |

†Survival derived from Kaplan–Meier analysis. ‡Hazard ratios (HRs), 95% confidence intervals (CIs) and their corresponding P values were calculated using univariate Cox proportional hazard models. Adeno, adenocarcinoma; aHR, adjusted hazard ratio; ECOG PS, Eastern Cooperative Oncology Group performance status; GP/GC, carboplatin or cisplatin plus gemcitabine; m, months; NPNC, carboplatin or cisplatin plus vinorelbine; OS, overall survival; P, Log-Rank P; SQC, squamous cell carcinoma; TNM, tumor node metastasis; TP/TC, carboplatin or cisplatin plus paclitaxel.
Table 5  Multivariate Cox regression analysis of prognostic factors for overall survival in pooled populations

| Variables                  | HR (95% CI) | P   |
|----------------------------|-------------|-----|
| Age (≤58 vs. >58)          | 1.25 (0.96–1.55) | 0.062 |
| TNM stage (IV vs. III)     | 1.30 (1.11–1.51) | 0.004 |
| Tumor histology            |             | 0.022 |
| Adeno                      | Ref.        |     |
| SQC                        | 1.22 (1.01–1.47) | 0.041 |
| Others                     | 1.38 (1.12–1.71) | 0.013 |
| P73 G4C14-to-A4T14         |             |     |
| Dominant                   | 0.82 (0.70–0.97) | 0.017 |
| Recessive                  | 0.75 (0.54–1.04) | 0.086 |

All of the variables yielding P values < 0.1 in the univariate analysis were used for multivariate Cox regression analysis. Adeno adenocarcinoma; CI, confidence interval; HR, hazard ratio; SQC squamous cell carcinoma; TNM, tumor node metastasis.

be considered with caution. Liu et al.’s study was the only one to investigate the association between the p73 G4C14-to-A4T14 polymorphism and the response of lung adenocarcinoma cell lines to chemotherapy. Nevertheless, the authors also considered that the negative results should be validated further in a prospective study with a larger group of patients. In our opinion, the in vitro assay may not be adequate to simulate the function of chemotherapy drugs in tumor patients. In our study, we found a significant correlation between the p73 G4C14-to-A4T14 polymorphism and survival outcomes in a Chinese population of advanced NSCLC patients treated with platinum-based chemotherapy. Patients with AT/AT and GC/AT genotypes had a more favorable response and better overall survival than those with the GC/GC genotype, which is consistent with previous published results. This may be explained by variation from the GC to AT allele, leading to the formation of a stem-loop structure, thus modulating the translation efficiency of p73 in tumors.

The present study has several strengths. We used two relatively large cohorts from four independent oncological departments for the discovery and validation of the association between the p73 G4C14-to-A4T14 polymorphism and clinical outcomes in advanced NSCLC patients. To ensure relatively homogeneous treatment, only those subjects who did not receive surgery and radiation therapy were enrolled. In addition, we also evaluated this polymorphism classified by models including genotypic, dominant,

Table 6  Association between p73 genotypes and OS stratified by selected variables

| Variables                  | GC/GC | GC/GC + AT/AT | aHR (95% CI)† |
|----------------------------|-------|---------------|---------------|
| Age (years)                |       |               |               |
| <58                        | 305 (60.5) | 19.93 (17.61–22.25) | 1.00 | 0.90 (0.72–1.11) | 0.305 |
| ≥58                        | 285 (60.9) | 15.07 (13.31–16.83) | 1.00 | 0.70 (0.56–0.86) | 0.001 |
| Gender                     |       |               |               |
| Male                       | 422 (61.2) | 15.97 (14.15–17.78) | 1.00 | 0.78 (0.66–0.94) | 0.007 |
| Female                     | 168 (59.6) | 19.27 (16.17–22.37) | 1.00 | 0.83 (0.55–1.18) | 0.125 |
| Smoking history             |       |               |               |
| Non-smokers                | 236 (58.4) | 19.07 (16.33–21.80) | 1.00 | 0.81 (0.64–1.02) | 0.078 |
| Smokers                    | 354 (62.3) | 15.90 (13.92–17.88) | 1.00 | 0.76 (0.62–0.93) | 0.006 |
| ECOG PS                    |       |               |               |
| 0–1                        | 535 (60.1) | 17.17 (15.44–18.90) | 1.00 | 0.77 (0.66–0.90) | 0.001 |
| 2                          | 55 (67.1)  | 17.77 (9.42–26.12)  | 1.00 | 0.93 (0.60–1.59) | 0.890 |
| Chemotherapy               |       |               |               |
| N/PNC                      | 208 (59.3) | 16.27 (13.06–19.48) | 1.00 | 0.89 (0.69–1.15) | 0.371 |
| GP/GC                      | 157 (58.4) | 17.47 (14.98–19.95) | 1.00 | 0.75 (0.56–1.01) | 0.055 |
| TP/TC                      | 185 (64.5) | 17.97 (14.41–21.52) | 1.00 | 0.79 (0.60–1.04) | 0.091 |
| DPDC                       | 40 (61.5)  | 18.40 (12.83–23.97) | 1.00 | 0.71 (0.37–1.38) | 0.313 |
| TNM stage                  |       |               |               |
| III                        | 237 (62.2) | 19.10 (16.81–21.40) | 1.00 | 0.79 (0.62–1.01) | 0.059 |
| IV                         | 353 (59.7) | 15.20 (13.18–17.22) | 1.00 | 0.77 (0.63–0.93) | 0.008 |
| Tumor histology            |       |               |               |
| Adeno                      | 370 (59.9) | 18.10 (16.02–20.19) | 1.00 | 0.74 (0.62–0.90) | 0.002 |
| SQC                        | 133 (62.4) | 14.57 (11.04–18.09) | 1.00 | 0.76 (0.55–1.06) | 0.108 |
| Others                     | 87 (61.7)  | 16.40 (12.90–19.90) | 1.00 | 0.95 (0.71–1.26) | 0.797 |

†Hazard ratios (HRs), 95% confidence intervals (CIs) and their corresponding P values were calculated using multivariate Cox proportional hazard models, adjusted for all clinical factors. ‡Survival derived from Kaplan–Meier analysis. Adeno, adenocarcinoma; aHR, adjusted hazard ratio; ECOG PS, Eastern Cooperative Oncology Group performance status; GP/GC, carboplatin or cisplatin plus gemcitabine; m, months; N/PNC, carboplatin or cisplatin plus vinorelbine; OS, overall survival; P<0.05; Log-Rank P, SQC, squamous cell carcinoma; TNM, tumor node metastasis; TP/TC, carboplatin or cisplatin plus paclitaxel.
Table 7  Correlations of p73 genotypes with chemotherapy efficacy in pooled populations

| Genotypes   | ORR (CR + PR) | DCR (CR + PR + SD) |
|-------------|--------------|--------------------|
|             | N (%) | $\chi^2$ | P* | OR (95% CI)† | P† | N (%) | $\chi^2$ | P* | OR (95% CI)† | P† |
| GC/GC       | 87 (14.8) | 4.272 | 0.039 | Reference | 468 (79.3) | 1.122 | 0.294 | Reference | 293 (76.7) | 0.92 | (0.64–1.45) | 0.374 |
| GC/AT+ AT/AT| 76 (19.9) | 0.69 | (0.41–0.93) | 0.047 | 2 test. |

P* values derived from $\chi^2$ test. †Odds ratios (ORs), 95% confidence intervals (CIs) and their corresponding P values were calculated using multivariate logistic regression analysis, adjusted for all clinical factors. CR, complete response; DCR, disease control rate; ORR, objective response rate; PR, partial response; SD, stable disease.

In conclusion, our findings indicated that the p73 G4C14-to-A4T14 polymorphism may be related to survival outcomes in advanced NSCLC patients following platinum-based chemotherapy. However, further studies are required to investigate the underlying mechanism by which this common p73 SNP affects outcomes in advanced NSCLC patients.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (No. 81572269), the Shanghai Health Bureau Foundation (No. 201440397) and the Med-Engineering Interdisciplinary Research Foundation of Shanghai Jiao Tong University (No. YG2015MS71).

Disclosure

No authors report any conflict of interest.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65: 5–29.
2. Shi Y, Sun Y. Medical management of lung cancer: Experience in China. Thorac Cancer 2015; 6: 10–6.
3. Chen X, Duan N, Zhang C, Zhang W. Survivin and tumorigenesis: Molecular mechanisms and therapeutic strategies. J Cancer 2016; 7: 314–23.
4. Kaghad M, Bonnet H, Yang A et al. Monoallelically expressed gene related to p53 at lp36, a region frequently deleted in neuroblastoma and other human cancers. Cell 1997; 90: 809–19.
5. Li G, Wang LE, Chamberlain RM, Amos CI, Spitz MR, Wei Q. p73 G4C14-to-A4T14 polymorphism and risk of lung cancer. Cancer Res 2004; 64: 6863–6.
6. Zhou X, Wu C. Association of p73 G4C14-A4T14 polymorphisms with genetic susceptibilities to breast cancer: A case-control study. Med Oncol 2012; 29: 3216–21.
7. Umar M, Upadhya R, Khurana R, Kumar S, Ghoshal UC, Mittal B. Role of p53 and p73 genes polymorphisms in susceptibility to esophageal cancer: A case control study in a northern Indian population. Mol Biol Rep 2012; 39: 1153–62.
8. Mittal RD, George GP, Mishra J, Mittal T, Kapoor R. Role of functional polymorphisms of P53 and P73 genes with the risk of prostate cancer in a case-control study from Northern India. Arch Med Res 2011; 42: 122–7.
9. Niwa Y, Hamajima N, Atsuta Y et al. Genetic polymorphisms of p73 G4C14-to-A4T14 at exon 2 and p53 Arg72Pro and the risk of cervical cancer in Japanese. Cancer Lett 2004; 205: 55–60.
10. De Feo E, Persiani R, La Greca A et al. A case-control study on the effect of p53 and p73 gene polymorphisms on gastric cancer risk and progression. Mutat Res 2009; 675: 60–5.
11. Lunghi P, Costanzo A, Mazzera L, Rizzoli V, Leverro M, Bonati A. The p53 family protein p73 provides new insights into cancer chemosensitivity and targeting. Clin Cancer Res 2009; 15: 6495–502.
12. Liu D, Wu C, Jiao Y et al. WEE1 kinase polymorphism as a predictive biomarker for efficacy of platinum-gemcitabine doublet chemotherapy in advanced non-small cell lung cancer patients. Sci Rep 2015; 5: 11114.
13. Eisenhauer EA, Therasse P, Bogaerts J et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 2009; 45: 228–47.
14. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature 2000; 408: 306–10.
15. Costanzo A, Merlo P, Pediconi N et al. DNA damage-dependent acetylation of p73 dictates the selective activation of apoptotic target genes. Mol Cell 2002; 9: 175–86.
16. Irwin MS, Kondo K, Marin MC, Cheng LS, Hahn WC, Kaelin WG Jr. Chemosensitivity linked to p73 function. Cancer Cell 2003; 3: 403–10.
17. Bergamaschi D, Gasco M, Hiller L et al. p53 polymorphism influences response in cancer chemotherapy via modulation of p73-dependent apoptosis. Cancer Cell 2003; 3: 387–402.
18 Frasca F, Vella V, Aloisi A et al. p73 tumor-suppressor activity is impaired in human thyroid cancer. Cancer Res 2003; 63: 5829–37.
19 Müller M, Schilling T, Sayan AE et al. TAp73/ΔNp73 influences apoptotic response, chemosensitivity and prognosis in hepatocellular carcinoma. Cell Death Differ 2005; 12: 1564–77.
20 Domínguez G, García JM, Peña C et al. Delta TAp73 upregulation correlates with poor prognosis in human tumors: Putative in vivo network involving p73 isoforms, p53, and E2F-1. J Clin Oncol 2006; 24: 805–15.
21 Bozzetti C, Nizzoli R, Musolino A et al. p73 and p53 pathway in human breast cancers. J Clin Oncol 2007; 25: 1451–3.
22 Carastro LM, Lin HY, Park HY et al. Role of p73 dinucleotide polymorphism in prostate cancer and p73 protein isoform balance. Prostate Cancer 2014; 2014: 129582.
23 Pfeifer D, Arbman G, Sun XF. Polymorphism of the p73 gene in relation to colorectal cancer risk and survival. Carcinogenesis 2005; 26: 103–7.
24 Lee KE, Hong YS, Kim BG et al. p73 G4C14 to A4T14 polymorphism is associated with colorectal cancer risk and survival. World J Gastroenterol 2010; 16: 4448–54.
25 Liu L, Wu C, Wang Y et al. Combined effect of genetic polymorphisms in P53, P73, and MDM2 on non-small cell lung cancer survival. J Thorac Oncol 2011; 6: 1793–800.
26 Liu K, Jiang L, Zhou X. Association of p73 G4C14-to-A4T14 polymorphism at exon 2 with the response of human lung adenocarcinoma cell lines to chemotherapy. Cell Biol Int 2010; 34: 185–8.