Associations of Polymorphisms in DNA Repair Genes and MDR1 Gene with Chemotherapy Response and Survival of Non-Small Cell Lung Cancer

Yan Du, Tong Su, Lijun Zhao, Xiaojie Tan, Wenjun Chang, Hongwei Zhang, Guangwen Cao

1 Department of Epidemiology, Second Military Medical University, Shanghai, China, 2 Department of Pulmonary Medicine, Changhai Hospital, Second Military Medical University, Shanghai, China

Abstract

Objectives: We aimed to determine the associations of genetic polymorphisms of excision repair cross-complementation group 1 (ERCC1) rs11615, xeroderma pigmentosum group D (XPD/ERCC2) rs13181, X-ray repair complementing group 1 (XRCC1) rs25487, XRCC3 rs1799794, and breast cancer susceptibility gene 1 (BRCA1) rs1799666 from the DNA repair pathway and multiple drug resistance 1 (MDR1/ABCB1) rs1045642 with response to chemotherapy and survival of non-small cell lung cancer (NSCLC) in a Chinese population.

Materials and Methods: A total of 352 NSCLC patients were enrolled to evaluate the associations of the six SNPs with response to chemotherapy and overall survival. Logistic regressions were applied to test the associations of genetic polymorphisms with response to chemotherapy in 161 advanced NSCLC patients. Overall survival was analyzed in 161 advanced and 156 early stage NSCLC patients using the Kaplan-Meier method with log-rank test, respectively. Multivariate Cox proportional hazards model was performed to determine the factors independently associated with NSCLC prognosis.

Results: BRCA1 rs1799666 minor allele C (TC+CC vs. TT, OR = 0.402, 95%CI = 0.204 – 0.794, p = 0.008) and MDR1/ABCB1 rs1045642 minor allele A (GA +AA vs. GG, OR = 0.478, 95%CI = 0.244 – 0.934, p = 0.030) were associated with a better response to chemotherapy in advanced NSCLC patients. Survival analyses indicated that BRCA1 rs1799666 TC+CC genotypes were associated with a decreased risk of death (HR = 0.617, 95% CI = 0.402 – 0.948, p = 0.028) in advanced NSCLC patients, and the association was still significant after the adjustment for covariates. Multivariate Cox regression analysis showed that ERCC1 rs11615 AA genotype (P = 0.020) and smoking (p = 0.037) were associated with increased risks of death in early stage NSCLC patients after surgery.

Conclusions: Polymorphisms of genes in DNA repair pathway and MDR1 could contribute to chemotherapy response and survival of patients with NSCLC.

Introduction

Lung cancer is one of the leading causes of cancer deaths worldwide [1]. Non-small-cell lung cancer (NSCLC) is the most common subtype and accounts for 85% of all lung cancer. Most NSCLC patients are at developed advanced tumor stage upon diagnosis and lose the opportunity of surgical resection [2]. Platinum-based combined chemotherapy is a standard treatment for advanced NSCLC. However, the outcome and survival of advanced NSCLC are generally poor, with a 5-year survival rate of only about 15%. In addition, the survival rate of advanced NSCLC varies greatly in different populations with diverse genetic background [3,4]. Furthermore, the therapeutic efficacy of platinum-based regimens was affected by drug resistance. Possible mechanisms of chemoresistance include alterations in drug efflux or influx, DNA repair capacity, and other cellular pathways required for response to DNA damage. For patients with early stage NSCLC that can be surgically excised, the long-term prognosis is not satisfactory and the 5-year survival rate after surgery is less than 50% [5]. The outcome of early stage NSCLC patients receiving surgical treatment is also closely related to individual genetic characteristics, since variation and expression...
levels of certain genes can affect the survival and adjuvant chemotherapy response [6,7]. Genetic variation analysis in candidate pathways has shown that an individual’s genetic background plays an important role in disease development, treatment response, and survival. For example, patients with completely resected NSCLC and negative expression of excision repair cross-complementation group 1 (ERCC1) protein in tumors benefit more from adjuvant cisplatin-based chemotherapy than those with ERCC1 positive expression [8]. Previous studies have shown that tumor-node-metastasis (TNM) staging system, age, performance status and weight loss are associated with NSCLC prognosis; however, the predictive powers of these factors are not optimal. Therefore, it is crucial to identify new biomarkers that can improve prognostic and predictive assessment accuracy to help developing personalized cancer treatment and patient-tailored chemotherapy, and eventually achieving better outcomes for NSCLC patients.

DNA repair capacity (DRC) is a double-edged sword in cancer etiology and treatment. Defects in DNA repair system drastically increase cancer risk [9]. On the other hand, DNA repair mechanism may reduce the therapeutic efficacy of chemotherapy by allowing cancer cells to fix DNA damages caused by these agents [10]. DNA repair involves coordination of many genes in four major DNA repair pathways, which are nucleotide excision repair (NER), base excision repair (BER), double-strand break repair (DSBR), and mismatch repair (MMR) pathways. Single nucleotide polymorphisms (SNPs) in DNA repair genes may modulate DNA repair capacity via influencing protein expression or activities, and therefore affecting lung cancer risk [11–13]. DNA repair gene polymorphisms may be associated with the risk and prognosis of NSCLC. Xeroderma pigmentosum group D (XPD/ERCC2) polymorphism can alter the secondary structure of mRNA [14]; and SNP in the X-ray repair cross-complementing group 1 (XRCC1) gene 5' untranslated region (UTR) greatly enhance trans-activator Sp1 binding element, therefore decreasing promoter activity and decreasing protein expression [15]. Furthermore, polymorphisms in these genes may also be potential prognostic markers for both chemotherapy response and overall survival of NSCLC patients [16–19].

Multiple drug resistance 1 (MDR1, also known as ABCC1) gene encodes P-glycoprotein (P-gp), a transmembrane transporter belonging to the ATP-binding cassette family, which can pump out intracellular chemotherapeutics and induce multiple drug resistance. MDR1/ABCC1 C3435T (rs1045642) polymorphism is associated with expression level and function of MDR1 [20], thus may affect the responses to anticancer drug treatment.

In this study, we selected six previously studied SNPs, located either on the protein coding regions or in regulatory regions of the genes, including ERCC1 rs11615, XPD/ERCC2 rs13181 and breast cancer susceptibility gene 1 (BRCA1) rs1799966 from the NER pathway; XRCC1 rs25487 from the BER pathway, XRCC3 rs1799794 from the DSBR pathway, as well as MDR1/ABCC1 rs1045642 (Table S1). We first investigated the associations between these SNPs and chemotherapy response in advanced NSCLC patients, then evaluated their relationships with the survival of NSCLC patients who received surgical treatment or chemotherapy.

Materials and Methods

Study population

The participants were recruited at Changhai Hospital of the Second Military Medical University. A total of 352 primary NSCLC patients diagnosed from July 1997 to October 2008 were enrolled (Dataset S1). All patients were histopathologically confirmed as NSCLC. Clinical and histopathologic data of patients were extracted from their medical records. All patients were of ethnic Han Chinese origin. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki (2000), and was approved by the Institutional Review Board of this university. All participants provided written informed consent.

Data collection and follow-up

Of the 352 NSCLC patients, 161 (45.7%) advanced NSCLC patients received a platinum-based chemotherapy. Besides cisplatin (DDP) or carboplatin (CBP), chemotherapy regimens also included gemcitabine (GEM), paclitaxel (TAX), docetaxel (DOX), vinorelbine (NVB), or pemetrexed disodium (PEM). Drug dosages were: DDP 75 mg/m² on day 1; CBP area under curve (AUC) = 5–6 g on day 1; GEM 1250 mg/m² on days 1 and 8; TAX 135–175 mg/m² on day 1 over 3 hours; DOX 75 mg/m² on day 1 over 1 hour; NVB 25 mg/m² on days 1 and 8; PEM 500 mg/m² on day 1. All drugs were administered by intravenous infusion every 3–4 weeks for a treatment cycle. Tumor response was assessed after 2 treatment cycles according to the Response Evaluation Criteria in Solid Tumors [21]. Responses were classified into complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD). Patients with CR, PR or SD were defined as “patients with clinical benefit”, and patients with PD were defined as “patients without clinical benefit” [22].

Follow-up was started 2 months after the definite diagnosis. Follow-up was performed by telephone or in-person interview at the outpatient department at an interval of 3 months according to our standard epidemiological procedure. Median follow-up period was 20.8 months (range: 1.0 to 178.6 months).

Genotyping

Genomic DNA was extracted from peripheral blood using QiAmp DNA extraction kits (Qiagen, Hilden, Germany). SNPs were genotyped using fluorescent-probe real-time quantitative PCR (qPCR) in a LightCycler™480 (Roche, Basel, Switzerland). Primers and probes (Taqman or Minor Groove Binder [MGB]) were designed and synthesized by GeneCore Biotechnologies (Shanghai, China). The sequences of primers and probes are available in Table S1. Each reaction mixture contained 0.2 μmol/L of primers, 0.2 μmol/L of probes, 0.1 μg-0.5 μg purified templates in Premix Ex Taq reaction system (Takara, Dalian, China). The reactions were programmed at 95°C for 10 s and followed by 40 cycles of 95°C for 10 s and 60°C for 30 s. All samples were successfully genotyped (Dataset S1). For quality control, 5% samples were randomly selected and directly sequenced, and 100% identical results were obtained.

Statistical analysis

Logistic regression analysis was performed to obtain odds ratios (ORs) and their 95% confidence intervals (95% CIs) for the relationships of genetic polymorphisms with chemotherapy response in 161 advanced NSCLC patients. Overall survival (OS) was analyzed using the Kaplan-Meier method in 161 advanced and 156 early stage NSCLC patients separately. Log-rank test was used to compare the survival curves. Forward stepwise multivariate Cox proportional hazards model (P enter = 0.05, P remove = 0.10) was performed to determine the factors contributing independently to NSCLC prognosis and estimate the hazard ratios (HRs) and their 95% CIs. Further subgroup analyses stratifying by histology, smoking status, Eastern Cooperative Oncology Group (ECOG) performance status, and stage were
also performed. Covariates were adjusted to obtain HRs and corresponding 95% CIs in subgroup analyses. All statistical tests were two-sided and conducted using Statistical Program for Social Sciences (SPSS 16.0 for Windows, SPSS, Chicago, IL). *P*, 0.05 was considered as statistically significant.

Results

Patient characteristics

The demographic and clinical characteristics of the patients are shown in Table 1. Of the 352 NSCLC cases, 156 (44.3%) were early stage patients who underwent surgical treatment, 161 (45.7%) were advanced patients at an inoperable stage and received platinum-based chemotherapy, 10 (2.8%) patients refused any treatment for personal reasons, and 25 (7.1%) patients lost to follow up after the first treatment at Changhai Hospital. Most (120/156, 76.9%) early stage NSCLC patients who underwent surgery received preoperative or postoperative adjuvant chemotherapy. Advanced NSCLC patients were more likely to receive radiation therapy compared to early stage patients (44.1% vs. 20.5%, *p*, 0.0001). Other demographic and clinical characteristics were similar between early stage and advanced NSCLC patients (*p*, 0.05 for all). Table S2 presented the genotype distributions of these six SNPs in different categories of the NSCLC patients.

Associations of SNPs with chemotherapy response

Of the 161 advanced NSCLC patients that received evaluable platinum-based chemotherapy, the number of patients with CR, PR, SD or PD was 0, 44, 65 and 52, respectively. The overall clinical benefit (CR + PR + SD) rate was 67.7% (109/161). *BRCA1* rs1799966, *MDR1/ABCB1* rs1045642 and clinical stage were

| Table 1. Demographic and clinical characteristics of the NSCLC patients. |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                            | **Total (n = 352)**          | **Classified according to treatment and follow-up results** |
|                            |                             | Early stage patients with surgery (n = 156) | Advanced patients with chemotherapy (n = 161) | Patients without treatment (n = 10) | Patients lost to follow up (n = 25) |
| Age (year)                 |                             |                                             |                                             |                                             |                                             |
| Range                      | 26–90                       | 26–84                                      | 30–90                                      | 56–76                                      | 35–78                                      |
| SD                         | 59.5±10.6                   | 59.2±9.9                                   | 58.9±11.3                                  | 66.7±7.8                                   | 62.5±9.6                                   |
| Sex, n (%)                 |                             |                                             |                                             |                                             |                                             |
| Male                       | 246(69.9)                   | 109(69.9)                                  | 108(67.1)                                  | 8(80.0)                                    | 21(84.0)                                   |
| Female                     | 106(30.1)                   | 47(30.1)                                   | 53(32.9)                                   | 2(20.0)                                    | 4(16.0)                                    |
| Histology, n (%)           |                             |                                             |                                             |                                             |                                             |
| Squamous cell carcinoma    | 118(33.5)                   | 54(34.6)                                   | 52(32.3)                                   | 4(40.0)                                    | 8(32.0)                                    |
| Adenocarcinoma             | 181(51.4)                   | 81(51.9)                                   | 82(50.9)                                   | 3(30.0)                                    | 15(60.0)                                   |
| Other                      | 53(15.1)                    | 21(13.4)                                   | 27(16.8)                                   | 3(30.0)                                    | 2(8.0)                                     |
| Stage, n (%)               |                             |                                             |                                             |                                             |                                             |
| I                          | 53(15.1)                    | 47(30.1)                                   | 0(0.0)                                     | 0(0.0)                                     | 6(24.0)                                    |
| II                         | 77(21.9)                    | 73(46.8)                                   | 0(0.0)                                     | 2(20.0)                                    | 2(8.0)                                     |
| IIIA                       | 48(13.6)                    | 36(23.1)                                   | 8(5.0)                                     | 1(10.0)                                    | 3(12.0)                                    |
| IIIB                       | 54(15.3)                    | 47(29.2)                                   | 3(10.0)                                    | 2(8.0)                                     |                                            |
| IV                         | 120(34.1)                   | 106(65.8)                                  | 2(10.0)                                    | 12(48.0)                                   |                                            |
| ECOG performance status    |                             |                                             |                                             |                                             |                                             |
| 0–1                        | 343(97.4)                   | 154(98.7)                                  | 155(96.2)                                  | 9(90.0)                                    | 24(96.0)                                   |
| ≥2                         | 9(2.6)                      | 2(1.3)                                     | 6(3.7)                                     | 1(10.0)                                    | 4(16.0)                                    |
| Smoking status             |                             |                                             |                                             |                                             |                                             |
| Never smokers              | 170(48.3)                   | 80(51.3)                                   | 73(45.3)                                   | 4(40.0)                                    | 13(52.0)                                   |
| Ever smokers               | 182(51.7)                   | 76(48.7)                                   | 88(54.7)                                   | 6(60.0)                                    | 12(48.0)                                   |
| <25 pack-years             | 54(15.3)                    | 27(17.3)                                   | 23(14.3)                                   | 1(10.0)                                    | 3(12.0)                                    |
| 25–50 pack-years           | 94(26.7)                    | 37(23.7)                                   | 46(28.6)                                   | 5(50.0)                                    | 6(24.0)                                    |
| >50 pack-years             | 34(9.7)                     | 12(7.7)                                    | 19(11.8)                                   | 0(0.0)                                     | 3(12.0)                                    |
| Radiation therapy          |                             |                                             |                                             |                                             |                                             |
| Never received             | 246(69.9)                   | 124(79.5)                                  | 90(55.9)                                   | -                                          | 22(88.0)                                   |
| Ever received              | 106(30.1)                   | 32(20.5)                                   | 71(44.1)                                   | -                                          | 3(12.0)                                    |

Abbreviations: ECOG, Eastern Cooperative Oncology Group; SD, stand deviation.

*P* values refer to the comparison between early stage patients with surgery and advanced patients with chemotherapy. Student’s t-test was used to compare age as a continuous variable; chi-square test was used to compare categorical variables, except for ECOG performance status, where Fisher's exact test was used.

†Comparisons between never smokers and ever smokers.

doi:10.1371/journal.pone.0099843.t001
significantlly associated with clinical benefit (Table 2). The patients carrying BRCA1 minor allele C (TG+CC) had significantly more chances of achieving clinical benefit than the patients carrying rs1799966 TT genotype (75.2% vs. 55.0%, OR = 0.402, 95% CI = 0.204–0.794, p = 0.008). Similarly, patients carrying MDR1/ABCB1 rs1045642 minor allele A (GA +AA) had more clinical benefit than patients carrying rs1045642 GG genotype (74.5% vs. 58.2%, OR = 0.478, 95% CI = 0.244–0.934, p = 0.030). In addition, the patients with clinical stage IV had significantly less chances of achieving clinical benefit than those with clinical stage III (61.3% vs. 80.0%, OR = 2.523, 95% CI = 1.171–5.437, p = 0.016). Furthermore, the associations continued to be significant in multivariate logistic regression analysis, which suggested that BRCA1 rs1799966 (TC+CC vs. TT), MDR1/ABCB1 rs1045642 (GA+AA vs. GG) and clinical stage (IV vs. III) were independent factors affecting response to chemotherapy (BRCA1 rs1799966: adjusted OR = 0.410, 95% CI = 0.203–0.831, adjusted p = 0.013; MDR1/ABCB1 rs1045642: adjusted OR = 0.488, 95% CI = 0.242–0.934, adjusted p = 0.045; clinical stage: adjusted OR = 2.698, 95% CI = 1.214–5.996, adjusted p = 0.015). No significant associations were observed between the other four SNPs (ERCC1 rs11615, XPD/ERCC2 rs13181, XRCC1 rs25487, XRCC3 rs1799794) or other characteristics (age, sex, histology, chemotherapy regimens, performance status, smoking status, and radiation therapy) and clinical benefit (p>0.05 for all).

Table 2. Associations of genotype and clinical stage with clinical benefit of chemotherapy among advanced NSCLC patients (N = 161).

| BRCA1 rs1799966 | CR+PR+SD, n (%) | PD, n (%) | OR (95% CI) | Adjusted P* | Adjusted OR* (95% CI) |
|-----------------|----------------|-----------|-------------|-------------|----------------------|
| TT              | 33 (55.0)      | 27 (45.0) | 1.00        | 1.00        |                      |
| TC+CC           | 76 (75.2)      | 25 (24.8) | 0.008       | 0.402       | (0.204–0.794)        |
| MDR1/ABCB1 rs1045642 |             |           |             |             |                      |
| GG              | 39 (58.2)      | 28 (41.8) | 1.00        | 1.00        |                      |
| GA +AA          | 70 (74.5)      | 24 (25.5) | 0.030       | 0.478       | (0.244–0.934)        |
| Clinical stage  |               |           |             |             |                      |
| III             | 44 (80.0)      | 11 (20.0) | 0.016       | 2.523       | (1.171–5.437)        |
| IV              | 65 (61.3)      | 41 (38.7) | 0.116       | 2.698       | (1.214–5.996)        |
| ERCC1 rs11615   |               |           |             |             |                      |
| GG              | 58 (63.7)      | 33 (36.3) | 1.00        | 1.00        |                      |
| GA+AA           | 51 (72.9)      | 19 (27.1) | 0.220       | 0.655       | (0.332–1.290)        |
| XPD/ERCC2 rs13181 |             |           |             |             |                      |
| TT              | 87 (68.0)      | 41 (32.0) | 1.00        | 1.00        |                      |
| TG+GG           | 22 (66.7)      | 11 (33.3) | 0.887       | 1.061       | (0.470–2.393)        |
| XRCC1 rs25487   |               |           |             |             |                      |
| CC              | 70 (66.8)      | 32 (31.4) | 1.00        | 1.00        |                      |
| CT+TT           | 39 (66.1)      | 20 (33.9) | 0.741       | 1.122       | (0.567–2.219)        |
| XRCC3 rs1799794 |               |           |             |             |                      |
| CC              | 39 (63.9)      | 22 (36.1) | 1.00        | 1.00        |                      |
| CT+TT           | 70 (70.0)      | 30 (30.0) | 0.425       | 0.760       | (0.387–1.493)        |

Abbreviations: CR, complete response; OR, odds ratio; PD, progressive disease; PR, partial response; SD, stable disease.

*Adjusted for BRCA1 rs1799966, MDR1/ABCB1 rs1045642 and clinical stage, adjusted ORs and P values were obtained from multivariate logistic regression analysis by forward stepwise method.
doi:10.1371/journal.pone.0099843.t002

Associations of SNPs with OS of patients with advanced NSCLC

Univariate analyses using Kaplan-Meier curves and log-rank tests indicated that BRCA1 rs1799966 (TG+CC vs. TT genotypes), radiation therapy (ever vs. never), and smoking status (never vs. ever) were significantly associated with an increased OS of advanced NSCLC patients with log-rank values of p = 0.042, 0.049, and 0.042, respectively (Fig. 1). The stepwise multivariate Cox analysis showed that BRCA1 rs1799966 (TG+CC vs. TT): HR = 0.617, 95% CI = 0.402–0.948, p = 0.028), radiation therapy (ever vs. never: HR = 0.611, 95% CI = 0.396–0.944, p = 0.027), and smoking (never vs. ever: HR = 0.574, 95% CI = 0.373–0.882, p = 0.011) independently predicted favorable survival of the advanced NSCLC patients. No significant associations were observed between the other SNPs or other characteristics and OS (log-rank p>0.05 for all).

We further examined the association of BRCA1 rs1799966 with survival in subgroup analyses by categorizing the patients according to histology, smoking status, ECOG performance status, and stage. In those with squamous cell carcinoma, those ever smoked, those with ECOG performance status of 1, or those at stage III, BRCA1 rs1799966 TT+CC genotypes predicted a longer OS than the TT genotype (log-rank p<0.05 for all) (Fig. 2). After adjusting for age, sex, histology, stage, ECOG performance status, smoking status, and radiation therapy in the Cox model, BRCA1 rs1799966 TT+CC genotypes were associated with a lower risk of death in patients with squamous cell carcinoma (HR = 0.324, 95% CI = 0.137–0.765, p = 0.010), patients ever smoked


Associations of SNPs with postoperative OS of patients with early stage NSCLC

*ERCC1* rs11615 and smoking status were statistically significantly associated with OS of early stage NSCLC patients (log-rank \( p = 0.009 \) and \( p = 0.013 \), respectively) (Fig. 3). In the stepwise multivariate Cox analysis, patients with *ERCC1* rs11615 AA genotype had an increased risk of death (deaths/cases: 5/9 for AA vs. 44/147 for GG+GA, HR = 3.087, 95% CI = 1.197–7.961, \( p = 0.020 \)). Smoking was also associated with worse survival (deaths/cases: 30/76 for ever smokers vs. 19/80 for never smokers, HR = 1.896, 95% CI = 1.040–3.455, \( p = 0.037 \)). We did not observe any significant associations of the other SNPs or other characteristics with OS (log-rank \( p > 0.05 \) for all).

Further stratification analyses in early stage NSCLC patients indicated that *ERCC1* rs11615 AA genotype predicted a significant poorer OS than GG+GA genotypes in patients with squamous cell carcinoma (log-rank \( p = 0.004 \)), those ever smoked (log-rank \( p = 0.018 \)), those with ECOG performance status of 1 (log-rank \( p = 0.007 \)), or those never receiving radiation therapy (log-rank \( p < 0.001 \)) (Fig. 4). After adjusting for age, sex, histology, stage, ECOG
performance status, smoking status, radiation therapy, and adjuvant chemotherapy in the Cox model. ERCC1 rs11615 AA genotype predicted a higher risk of death in early stage patients with squamous cell carcinoma (HR = 6.633, 95% CI = 1.184–37.164, p = 0.031), those ever smoked (HR = 3.324, 95% CI = 1.040–10.627, p = 0.043), those with ECOG performance status of 1 (HR = 2.835, 95% CI = 1.063–7.560, p = 0.037), or those never receiving radiation therapy (HR = 5.381, 95% CI = 1.857–15.593, p = 0.002).

Discussion

In this study, we evaluated relationships between six SNPs with chemotherapy response and survival of NSCLC in a Han Chinese population. We observed that the minor allele C of BRCA1 rs1799966 from the DSBR pathway was associated with a better treatment response and a longer survival in advanced NSCLC patients underwent platinum-based chemotherapy. In addition, minor allele A of MDR1/ABCB1 rs1045642 was significantly associated with clinical benefit of chemotherapy in advanced
NSCLC patients. For early NSCLC patients, carriers of ERCC1 rs11615 AA genotype had an increased risk of death.

BRCA1 gene belongs to the NER system [23], which is the major repair system that reduces platinum-induced DNA damage. BRCA1 involves in many activities including homologous recombination, nonhomologous end joining, and mismatch repair [24]. BRCA1 protein is a nuclear phosphoprotein with multiple roles not only in DNA damage repair but also in cell cycle checkpoint or cell death machinery [24,25]. In vitro studies have reported that BRCA1 can regulate chemotherapy agent sensitivity; for example, the absence of BRCA1 causes high sensitivity to cisplatin [24]. BRCA1 expression is a reliable indicator of chemoresistance in NSCLC patients receiving treatment by DNA-damaging agents such as platinum [26–28]. Patients with lower BRCA1 expression have better survival in platinum-based neoadjuvant chemotherapy [26–28], which is further confirmed by a recent meta-analysis [29]. Genetic polymorphisms may affect the protein expression, structure, and/or function; however, previous studies have rarely investigated the associations between BRCA1 polymorphisms with NSCLC survival. One study in a Korean population reported no association between BRCA1 rs1799966 and NSCLC survival; however, they did find BRCA1 haplotype AACC (rs1799966-rs8176199-rs8067269-rs2070833) predicted shorter survival among advanced NSCLC patients receiving platinum-based chemotherapy [18]. In our analysis, we observed BRCA1 rs1799966 minor allele C was significantly associated with better chemotherapy response and longer survival in advanced NSCLC patients. rs1799966 is a nonsynonymous SNP in the coding region of the COOH-terminal domain, termed as BRCT, which is essential for homologous recombination in repairing DNA double-stranded breaks [30]. The amino acid change from proline to leucine at position 871 of rs799917 leads to a non-conservative change of the unique structural properties of the BRCA1 protein. In addition, this SNP is in linkage disequilibrium with rs799917, which lies in the middle of a strongly conserved region of the BRCA1 gene [31].

ERCC1 is another important enzyme in the NER pathway of DNA repair, and is responsible for the 5′ incision of damaged DNA [32]. Previous studies have demonstrated that ERCC1 expression is related to clinical benefit of platinum-based chemotherapy [5,8,33,34], and could be used as a biomarker for NSCLC treatment. The variant genotype in ERCC1 polymorphisms may destroy or alter the repair functions possibly through changing the expression levels. rs11615 is a synonymous SNP located in codon 118 of the ERCC1 gene, which encodes the amino acid asparagines. The variant allele of rs11615 may results in altered mRNA level [35]. Studies conducted in different populations showed that the GG genotype of ERCC1 rs11615 was associated with a better survival in advanced NSCLC patient [36,37]. In contrary, a study conducted in Japan did not find significant associations between ERCC1 rs11615 and survival [38]. Furthermore, a meta-analysis including 17 studies could not confirm the association of ERCC1 rs11615 with survival, neither in the overall nor ethnic stratified analyses [39]. The inconsistent results of previous studies may be due to ethnic differences, the stage of the disease, and/or therapeutic variations. Few studies ever investigated the association between rs11615 and survival of early stage NSCLC. In the present study, we detected that the AA genotype of rs11615 was associated with poorer OS in early stage NSCLC patients. One important meta-analysis concluded that high ERCC1 was associated with significantly worse OS in platinum-treated NSCLC patients [40]. These evidences indicate that the variant allele genotype (AA) of rs11615 is related to high expression of ERCC1. However, the effects of radiation therapy are complex. A fraction of our early stage NSCLC patients (32/156, 20.5%) received radiation therapy, thus we cannot rule out the possible influence of radiation therapy on the association of ERCC1 rs11615 with survival.

One reason of treatment failure in cancer patients is drug resistance, which can be influenced by factors affecting the efflux and influx of chemotherapeutics across the cell membrane. MDR1/ABCB1 is responsible for the efflux of many chemotherapeutics through the cell membrane by hydrolysis of ATP [41]. rs1045642 has a G>A change at cDNA position 3435 in exon 26 and plays a role in the P-gp function [20]. However, results from studies of MDR1/ABCB1 rs1045624 and platinum-based chemo-

Figure 3. Factors associated with overall survival of early stage NSCLC patients. (A) ERCC1 rs11615, (B) smoking status.

doi:10.1371/journal.pone.0099843.g003
therapy response of NSCLC are inconsistent in different populations [36,42]. Recent meta-analyses confirmed this SNP was associated with chemotherapy response in overall and Asian populations [39,43]. However, we obtained opposite results from the meta-analysis findings, showing \textit{MDR1}/\textit{ABCB1} variant allele carriers had more clinical benefits of chemotherapy. The discrepancy may possibly be explained by potential confounders such as radiation therapy.

Not surprisingly, smoking was associated with NSCLC prognosis in both early and advanced stage patients. Smoking causes impaired lung function, and is the well established risk factor of NSCLC occurrence and poor prognosis. Smoking can cause DNA damages such as DNA adducts and double-strand DNA break lesions [44]. It can also interfere with DNA repair function, thus greatly affecting patient outcomes.

The current study has several limitations. First are the relatively small sample size and a short follow-up period, especially in subgroup analyses, therefore raising the issue of false positive findings. Our results did not reach statistical significance after correcting for multiple comparisons, and these findings need to be

![Figure 4. Subgroup analysis results of overall survival in early stage NSCLC patients according to \textit{ERCC1} genotypes (GG+GA vs. AA).](image)

(A) Squamous cell carcinoma group, (B) ever smokers group, (C) ECOG performance status = 1 group, (D) never receiving radiation therapy group. Number in parenthesis, number of deaths/number of cases.

doi:10.1371/journal.pone.0099843.g004
further validated. Second, we collected OS data instead of disease-specific survival since it is difficult to confirm the real cause of death in some patients. Third, we only selected limited number of SNPs based on their functions, while other polymorphisms in these genes may affect NSCLC chemotherapy response and prognosis. Last, NSCLC is a heterogeneous group of neoplasm, and multiple mechanisms may determine the response and prognosis, we were unable to account all factors in the current analysis.

To conclude, our study provided further evidences that polymorphisms in DNA repair pathway genes and MDR1 gene could influence treatment response and survival of NSCLC patients. The mechanisms of how the differences in SNP nucleotide sequences or tandem repeats can affect gene transcription remain largely unclear. Further large-scale studies are needed to elucidate the functions and mechanisms that may help in improving the outcomes of NSCLC patients.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, et al. (2011) Global cancer statistics. CA Cancer J Clin 61: 69–90.
2. Wu L, Chang W, Zhao J, Yu Y, Tan X, et al. (2010) Development of autoantibody signatures as novel diagnostic biomarkers of non-small cell lung cancer. Clin Cancer Res 16: 3760–3769.
3. Sekine I, Yamamoto N, Nishio K, Saijo N (2000) Emerging ethnic differences in lung cancer therapy. Br J Cancer 99: 1757–1762.
4. Siegel R, Naishadham D, Jemal A (2012) Cancer statistics, 2012. CA Cancer J Clin 62: 10–29.
5. Arrigada R, Bergman B, Dunant A, Le Chevalier T, Pigoun JP, et al. (2004) Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small cell lung cancer. N Engl J Med 350: 351–360.
6. Yoo SS, Choi JE, Lee WK, Choi YY, Kam S, et al. (2009) Polymorphisms in the GASPASE gene and survival in patients with early-stage non-small-cell lung cancer. J Clin Oncol 27: 5823–5829.
7. Park JY, Lee WK, Jung DK, ChoiJE, Park TL, et al. (2009) Polymorphisms in the FAS and FASL genes and survival of early stage non-small cell lung cancer. Cancer 116: 1794–1800.
8. Olausen KA, Dunant A, Fournet P, Brammilla E, Andre F, et al. (2006) DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med 355: 963–971.
9. Wei Q, Feaster ML, Levin B (2000) DNA repair: a double-edged sword. J Natl Cancer Inst 92: 440–441.
10. Rosell R, Lord RV, Tarom M, Reguart N (2002) DNA repair and cisplatin resistance in non-small-cell lung cancer. Lung Cancer 38: 217–227.
11. Taron M, Lord RV, Felip E, Mendez P, Souglakos J, et al. (2004) BRCA1 functions as a differential modulator of chemotherapy-induced apoptosis. Cancer Res 63: 6221–6228.
12. Zienolddiny S, Campa D, Lind H, Ryberg D, Skaug V, et al. (2006) Polymorphisms in DNA repair genes and risk of non-small-cell lung cancer: a population-based study. Cancer Epidemiol Biomarkers Prev 15: 1794–1800.
13. Zhang X, Miao X, Liang G, Hao B, Wang Y, et al. (2005) Polymorphisms in the FAS and FASL genes and survival in patients with early-stage non-small-cell lung cancer. Cancer 116: 1794–1800.
40. Hubner RA, Riley RD, Billingham LJ, Popat S (2011) Excision repair cross-complementation group 1 (ERCC1) status and lung cancer outcomes: a meta-analysis of published studies and recommendations. PLoS One 6:e25164.

41. Campa D, Sainz J, Pardini B, Vodickova L, Naccarati A, et al. (2012) A comprehensive investigation on common polymorphisms in the MDR1/ABCB1 transporter gene and susceptibility to colorectal cancer. PLoS One 7: e32784.

42. Vinolas N, Provencio M, Reguart N, Cardenal F, Alberola V, et al. (2011) Single nucleotide polymorphisms in MDR1 gene correlates with outcome in advanced non-small cell lung cancer patients treated with cisplatin plus vinorelbine. Lung Cancer 71: 191–198.

43. Yin JY, Huang Q, Zhao YC, Zhou HH, Liu ZQ. (2012) Meta-analysis on pharmacogenetics of platinum-based chemotherapy in non small cell lung cancer (NSCLC) patients. PLoS One 7:e38150.

44. Albino AP, Jorgensen ED, Rainey P, Gillman G, Clark TJ, et al. (2009) gammaH2AX: A potential DNA damage response biomarker for assessing toxicological risk of tobacco products. Mutat Res 678: 43–52.