The Association between COX-2 Polymorphisms and Hematologic Toxicity in Patients with Advanced Non-Small-Cell Lung Cancer Treated with Platinum-Based Chemotherapy

Fei Zhou, Guanghui Gao, Shengxiang Ren, Xuefei Li, Yayi He, Caicun Zhou*

Department of Oncology, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Tongji University Cancer Institute, Shanghai, China

Abstract

Background and Objective: Overexpression of COX-2 is proved to contribute to tumor promotion and carcinogenesis through stimulating cell proliferation, inhibiting apoptosis and enhancing the invasiveness of cancer cells. Apoptosis-related molecules are potential predictive markers for survival and toxicity in platinum treatment. This study aimed at investigating the association between COX-2 polymorphisms and the occurrence of grade 3 or 4 toxicity in advanced non–small cell lung cancer patients treated with platinum-based chemotherapy.

Materials and Methods: Two hundred and twelve patients with inoperable stage IIIb-IV NSCLC received first-line chemotherapy between 2007 and 2009 were recruited in this study. Four functional COX-2 polymorphisms were genotyped by PCR-based restriction fragment length polymorphism (RFLP) methods.

Results: The incidence of grade 3 or 4 hematologic toxicity was significantly higher in G allele carriers of the COX-2 rs689466 (−1195G/A) polymorphism compared with wild-type homozygotes AA (P value = 0.008; odds ratio, 2.47; 95% confidence interval, 1.26–4.84) and the significance still existed after the Bonferroni correction. Statistically significant difference was also found in grade 3 or 4 leukopenia (P value = 0.010; OR = 2.82; 95%CI = 1.28–6.20). No other significant association was observed between genotype and toxicity in the study. The haplotype analysis showed that the haplotype AGG was associated with a reduced risk of grade 3 or 4 hematologic and leukopenia toxicity (P value = 0.009; OR = 0.59; 95%CI = 0.39–0.88 and P value = 0.025; OR = 0.61; 95%CI = 0.39–0.94, respectively) while the haplotype GGG was associated with an increased risk of grade 3 or 4 hematologic and leukopenia toxicity (P value = 0.009; OR = 1.71; 95%CI = 1.14–2.56 and P value = 0.025; OR = 1.65; 95%CI = 1.06–2.57, respectively).

Conclusion: This investigation for the first time suggested that polymorphism in COX-2 rs689466 may be a potent biomarker in predicting severe hematologic toxicity in NSCLC patients after platinum-based chemotherapy.

Introduction

Lung cancer is the most commonly diagnosed cancer and the leading cause of cancer-related death in the world and NSCLC comprises the most common form of it [1–2]. Most NSCLC patients diagnosed are in the advanced stages, with the majority of whom presenting with stage III or IV disease. 5-year survival of these patients is still disappointingly low at less than 20% [2].

Platinum-based regimens have been used as the standard first-line chemotherapy in NSCLC patients [3–4] while the unpredictable and occasionally serious side effects, especially hematologic toxicity, continue to be an intractable problem. The incidence and severity of toxicities vary greatly between individuals [5]. Thus, searching of predictive markers that can identify patients who will benefit significantly from chemotherapy with minimal toxicity is a necessary and promising job in lung cancer research.

Most platinum compounds induce damage to tumors through induction of apoptosis while apoptosis is responsible for the characteristic hematologic toxicity, gastrointestinal toxicity, and most other drug toxicities [6]. It also suggests that the development of platinum compounds resistance could be the result of either inhibition of apoptotic genes or activation of antiapoptotic genes. Tumors that are resistant to cisplatin might also become resistant to the induction of programmed cell death as a consequence of the development of survival mechanisms during malignant transformation [7]. Therefore, apoptosis-related molecules are potential predictive markers for survival and toxicity in platinum-based treatment. Recently, caspase-3(CASP3), an apoptosis-related gene, was reported to be associated with severe hematologic toxicity risk [6].
Cyclooxygenase-2 (COX-2), also known as prostaglandin-endoperoxide synthase 2 (PTGS2), is a key enzyme involved in cancer development and progression and plays an important role in the modulation of apoptosis, angiogenesis, immune response, and tumor invasion [9–11]. COX-2 overexpression shows reduced apoptotic susceptibility by up-regulation of Bcl-2 and suppression of CASP3 and CASP9, two important families of apoptosis-related molecules [10–11]. It is reported that COX-2 is overexpressed in various malignancies such as gastric carcinoma, esophagus carcinoma, including NSCLC, suggesting its involvement in pulmonary tumorigenesis [12–14]. Increased COX-2 expression is also associated with more aggressive tumor behavior and poorer prognosis in NSCLC patients [15]. Preclinical study shows that taxanes may stimulate the expression of COX-2 gene and decrease the efficacy of anti-cancer and explain, at least partly, the toxicity of these drugs [16]. Additionally, overexpression of COX-2 mRNA is related to ionizing radiation (IR) induced pulmonary inflammation and inhibiting the IR-induced COX-2 expression could be helpful against radiation-induced normal tissue injury [17].

Several functional single nucleotide polymorphisms (SNPs) that have been identified in the COX-2 gene may contribute to different gene expression or enzyme activities [18–19]. A recent study shows that COX-2 gene polymorphism may be a potential predictive marker for survival in locally advanced NSCLC patients treated with chemoradiotherapy or radiotherapy alone [20]. Although the associations between genetic polymorphisms of COX-2 and the risk of developing certain cancers [14,19,21,22] and survival outcome have been reported [20], similar studies with toxicity have rarely been reported.

In this study, four putative functional SNPs in COX-2 gene were investigated. These SNPs include rs689465 (−1290A/G), rs689466 (−1195G/A), and rs20417 (−765G/C) in the promoter region, which were demonstrated to influence the expression of COX-2 [14,23]; rs3218625 (−1759G/A) in exon10, which was also associated with increased risk of gastric cardia adenocarcinoma [24]. Using DNA samples obtained from a series of patients with advanced NSCLC treated with platinum-based chemotherapy, we assessed the association between these COX-2 polymorphisms and toxicity outcomes.

### Materials and Methods

#### Patient recruitment and follow up

Patients with newly diagnosed advanced stage lung cancer were enrolled in the study at Shanghai Pulmonary Hospital in Shanghai, China, between January 2007 and December 2009. Patients with histological confirmation of NSCLC stage IIIIB and stage IV were selected. Other eligibility criteria included Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–2; aged above 18 years; adequate hematologic function (hemoglobin >9 g/dl, neutrophil count >1500/mm³, and platelet count >100 000/mm³); adequate renal function (creatinine clearance rate >50 ml/sec); adequate liver function (bilirubin <1.5 times the normal upper limit, aspartate aminotransferase and alanine aminotransferase <2 times the normal upper limit) and measurable disease. Patients with symptomatic brain metastases, spinal cord compression, uncontrollable massive pleural effusion and those who previously received chemotherapy were excluded. The protocol was conducted according to the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the ethics committees of Tongji University Affiliated Shanghai Pulmonary Hospital. The informed consent was written and obtained from each patient before the initiation of any study related procedure. This was a prospectively planned study and biological samples and later the study was designed.

Clinical data were systematically recorded at entry. Before starting any treatment, all patients underwent a complete medical history interview, physical examination and laboratory testing, including routine hematology and biochemistry analyses, staging with chest radiographs and computed tomography of the thorax and abdomen, and magnetic resonance imaging of the brain and a bone scan.

The incidence of grade 3 or 4 toxicity was assessed twice a week during cycle 1 chemotherapy, then the assessment was repeated once at the beginning of every cycle or before chemotherapy for day 8 treatment according to the National Cancer Institute Common Toxicity Criteria version 3.0 (CTCAE 3.0). Patient charts were reviewed to extract data on toxicities experienced during first-line chemotherapy. The worst toxicity grade of each patient in all the chemotherapy cycles was recorded. The investigators were blinded to the polymorphism status of the patients.

### Chemotherapy Regimens

All 212 patients enrolled in the study were inoperable and were given first-line platinum-based chemotherapy: cisplatin at a dose of 75 mg/m² or carboplatin (AUC = 5) given on day 1 combined with either gemcitabine at a dose of 1000 mg/m² on days 1 and 8, or vinorelmine 25 mg/m² on days 1 and 8, or paclitaxel 175 mg/m² on day 1, or docetaxel 75 mg/m² on day 1, for a maximum of six cycles, up to disease progression or unacceptable toxicity. The cycles were repeated every three weeks and all chemotherapeutic drugs were administered i.v.

Dose modification was according to the NCCN guideline and it was done by protocol. Briefly, if toxicity higher than grade 3 non-hematology toxicity (except for nausea and vomiting) and grade 4 hematology toxicity, neutropenia without febrile lasting more than 7 days, or febrile neutropenia or infection and/or thrombocytopenia associated with bleeding occurs, the dose of the cytotoxic agents in the next cycle was reduced by 25%. Concomitant supportive therapies, such as erythropoietic agents or granulocyte colony-stimulating factors, were allowed according to the American Society of Clinical Oncology guidelines [25].

### DNA collection and genotyping

Genomic DNA was extracted from 5 mL blood sample that was collected from each patient upon recruitment. Genotypes were determined by PCR-based restriction fragment length polymorphism (RFLP-PCR). The PCR primer pairs used to amplify COX-2 promoter region containing rs689465 (−1290A/G), rs689466 (−1195G/A), rs20417 (−765G/C) and rs3218625 (−1759G/A). The sequences of primers are as followed: 1290F5'-caggttttagctgtcatttcg-3', 1195G5'-ctcggagagga-3', 1195F5'-cctcggagactacttctgc-3', 1290F5'-gctcggagagaactggc-3', 765F5'-tatttcgaagattacccctgc-3' / 765R3'-gtaatttcgtaaccaaatg-5' / 1759F5'-gtgtctctgcta-3' / 1759R5'-ctgtcggagagga-3'.

The primers and reaction conditions were described in our previous report [26]. Genotypes were confirmed by direct DNA sequencing of the PCR products. A 10% blind, random sample of study subjects was genotyped twice by different persons and the reproducibility was 100%.

### Statistics analysis

Toxicity outcome in each group was dichotomized by the presence or absence of grade 3 or 4 toxicity during the first-line treatment. The associations between each genetic polymorphism...
and grade 3 or 4 toxicity were estimated by odds ratios (OR) and their 95% confidence intervals (95%CI), which were calculated by unconditional logistic regression. Adjusting covariates were performance status, gender, smoking status, histology, stage, radiation therapy, the number of cycles of chemotherapy received during first-line treatment, the receipt of taxanes and the type of platinum of agent (cisplatin versus carboplatin). Tests for trend were done by including genotypes as an ordinal variable in regression models. Hardy-Weinberg equilibrium was tested by Pearson Chi-Square test ($X^2$). Allele and genotype frequencies, Hardy-Weinberg equilibrium, linkage disequilibrium analysis, haplotypes and their frequencies were conducted online using SHEsis software platform, which is available at http://analysis.bio-x.cn [27,28]. For each gene, the Bonferroni correction was made for $p$ value. All $p$ values reported were two-sided, and a level of 0.05 was considered statistically significant. All statistical analyses used SPSS, version 17.0.

Results

Patient characteristics and toxicity outcomes

A total of 212 patients with advanced stage NSCLC were enrolled in this study. The median age at diagnosis was 60 years (range, 33–77 years). Of the subjects, 153 (72.1%) were male. All patients had advanced inoperable tumors, in which 73 (34.4%) with stage IIIb, and 139 (65.6%) with stage IV disease. According to the cancer cell types, adenocarcinoma was the most common histology (n = 117, 55.2%), followed by 62 (29.2%) squamous cell carcinoma and 33 (15.6%) adenosquamous carcinoma. All patients had an ECOG performance status of 1 or 0. There were 120 (56.6%) smokers and 92 (43.4%) non-smokers. No patient has had an ECOG performance status of 1 or 0. There were 120 (56.6%) smokers and 92 (43.4%) non-smokers. No patient has had an ECOG performance status of 1 or 0.

Eighty-two patients (38.7%) suffered from grade IIIb, and 139 (65.6%) with stage IV disease. According to the cancer cell types, adenocarcinoma was the most common histology (n = 117, 55.2%), followed by 62 (29.2%) squamous cell carcinoma and 33 (15.6%) adenosquamous carcinoma. All patients had an ECOG performance status of 1 or 0. There were 120 (56.6%) smokers and 92 (43.4%) non-smokers. No patient has had an ECOG performance status of 1 or 0. There were 120 (56.6%) smokers and 92 (43.4%) non-smokers. No patient has had an ECOG performance status of 1 or 0.

Table 1. Clinical characteristics of NSCLC patients.

| Patient characteristics | Number (%) |
|-------------------------|------------|
| Total no. patients | 212 |
| Median age (years) | 60 (33–77) |
| Gender | |
| Male | 153 (72.1) |
| Female | 59 (27.9) |
| Smoking status | |
| Non-smoker | 92 (43.4) |
| Smoker | 120 (56.6) |
| Histological cell type | |
| Adenocarcinoma | 117 (55.2) |
| Squamous cell | 62 (29.2) |
| Adenosquamous carcinoma | 33 (15.6) |
| Stage | |
| IIIb | 73 (34.4) |
| IV | 139 (65.6) |
| Radiation therapy | |
| Yes | 38 (17.9) |
| No | 174 (82.1) |
| Chemotherapy | |
| GP | 133 (62.7) |
| GC | 17 (8.1) |
| NP | 26 (12.3) |
| NC | 16 (7.5) |
| T/P+P | 7 (3.3) |
| T/P+C | 13 (6.1) |
| The cycles of regimens received | |
| 1 | 3 (1.4) |
| 2 | 63 (29.7) |
| 3 | 28 (13.2) |
| 4 | 108 (50.9) |
| 5–6 | 10 (4.7) |

GP = gemcitabine + cisplatin. GC = gemcitabine + carboplatin. NP = vinorelbine + cisplatin. NC = vinorelbine + carboplatin. T/P+ = docetaxel/paclitaxel + cisplatin. T/P+ C = docetaxel/paclitaxel + carboplatin.

*PS, Performance Status.

The COX-2 SNPs and Toxicity in Patients with NSCLC

For the COX-2 A/G polymorphism at rs689465, 178 (84.0%) patients were homozygous of the A/A genotype, whereas 31 (14.6%) were heterozygous A/G and 3 (1.4%) were variant homozygotes G/G. For the COX-2 rs689466 polymorphism, 67 (31.6%) patients were homozygous of the A/A genotype, whereas 99 (46.7%) were heterozygous A/G and 46 (21.7%) were variant homozygotes G/G. For the COX-2 rs3218625 polymorphism, 207 (97.6%) patients had the reference G/G genotype, whereas 5 (2.4%) were A/G. For the COX-2 rs20417 polymorphism, 195 (92.0%) patients were homozygous of the G/G genotype, whereas 16 (7.5%) were heterozygous A/G and 1 (0.5%) were variant homozygotes C/C. All the genotype distributions were in Hardy-Weinberg equilibrium (P $>$ 0.05).

All patients received platinum-based chemotherapy. 150 (70.8%) received gemcitabine plus cisplatin/carboplatin regimens (GP or GC), 42 (19.8%) received vinorelbine plus cisplatin/carboplatin (NP or NC) and 20 (9.4%) were given docetaxel/pacli taxel plus cisplatin/carboplatin (DP or DC; PP or PC). There was no significant association between the distribution of treatment regimens and polymorphism group. Table 2 shows the detailed distribution of treatment regimen by polymorphism group.

All chemotherapy-related toxicities were recorded in each treatment cycle. Incidences of all grade 3 and 4 toxicities were shown in Table 3. Eighty-two patients (38.7%) suffered from grade 3 or 4 hematologic toxicity, of whom 58 (27.4%) had grade 3 or 4 leukopenia, 26 (12.3%) grade 3 or 4 thrombocytopenia, 8 (3.8%) grade 3 or 4 anemia. Twenty-eight (13.0%) patients experienced grade 3 or 4 gastrointestinal toxicity. Additionally, Twenty-three patients (10.8%) and ten patients (4.6%) suffered from alopecia (any grade) and 3 or 4 cardiac toxicity, respectively. There were no patients who experienced more than one category of the toxicities.

Association between COX-2 polymorphisms and grade 3 or 4 toxicity

Logistic regression was carried out to reveal the association between COX-2 polymorphisms and patient outcomes. The association between polymorphisms and toxicity was shown in Table 4.
The incidence of grade 3 or 4 hematologic toxicity (44.8%) was significantly higher in G allele carriers of the COX-2 rs689466 polymorphism (P = 0.008; OR = 2.47; 95%CI = 1.26–4.84; the significance remained after the Bonferroni correction (P = 0.024); Bold in Table 4) compared to those with wild-type homozygotes AA (25.4%). When only severe leucopenia toxicity was considered, statistically significant difference was also found in rs689466 polymorphism carried with the frequency of different genotypes and occurrence of grade 3 or 4 leucopenia (P = 0.010; OR = 2.82; 95%CI = 1.28–6.20; the significance remained after the Bonferroni correction (P = 0.030); Bold in Table 4). Analysis of grade 3 or 4 thrombocytopenia or anemia toxicity revealed no statistically significant association with rs689466 polymorphism. No other significant association between genotype and non-hematologic toxicity was observed in this polymorphism.

It should be noted that the variant AG and AA genotypes of rs3218625 polymorphism showed a tendency to be associated with high risk of anemia compared with the GG genotype (P = 0.045; OR = 16.55; 95%CI = 0.67–86.75). However, the significance lost after the Bonferroni correction (P = 0.135, Table 4). There was no other significant association between the risk of any grade 3 or 4 toxicity and rs689465 and rs20417 polymorphisms (Table 4).

**Discussion**

In the present study, we investigated whether polymorphisms of COX-2 were associated with increased toxicity in advanced NSCLC patients treated with platinum-based chemotherapy. We found that patients carrying at least one variant COX-2 rs689466 G allele (AG or GG) were associated with a significantly increased risk of grade 3 or 4 hematologic and leukopenia toxicity (P = 0.009; OR = 0.59; 95%CI = 0.39–0.88 and P = 0.025; OR = 0.61; 95%CI = 0.39–0.94, respectively), while the haplotype GGG was associated with an increased risk of grade 3 or 4 hematologic and leukopenia toxicity (P = 0.009; OR = 1.71; 95%CI = 1.14–2.56 and P = 0.025; OR = 1.65; 95%CI = 1.06–2.57, respectively). No significant association between other haplotypes and 3 or 4 toxicities was observed (Table 5).

Recent studies suggest that SNPs in COX-2 promoter may alter the enzyme function of COX-2 by differential regulation of COX-2 expression. The rs689466 polymorphism locates in the COX-2 promoter region, which contains several key cis-acting regulatory elements and has decisive roles in the regulation of COX-2 transcription [23,33]. COX-2 rs689466 could affect gene expression and fibrosis when given with radiation [31,32]. However, in the present study, we investigated whether polymorphisms of COX-2 were associated with increased toxicity in advanced NSCLC patients treated with platinum-based chemotherapy. We found that patients carrying at least one variant COX-2 rs689466 G allele (AG or GG) were associated with a significantly increased risk of grade 3 or 4 hematologic and leukopenia toxicity.

**COX-2** is an inducible form of the enzyme and expresses primarily in response to inflammatory stimuli like cytokines or growth factors and mediates the production of prostaglandins that support the inflammatory process [13]. In relation to radiation-induced oral mucositis, it has been demonstrated that COX-2 expression in hamsters increased their response to targeted radiation after radiation injury [29]. The expression of COX-2 is also associated with radiation-induced small bowel injury [30] and pulmonary inflammation [17]. In addition, selective COX-2 inhibitor may protect normal tissues by reducing acute inflammation and fibrosis when given with radiation [31,32]. However, the association between the expression of COX-2 and chemotherapy-induced toxicity has not been reported yet.

The COX-2 SNPs and Toxicity in Patients with NSCLC

The COX-2 SNPs and Toxicity in Patients with NSCLC

**Haplotype analysis**

Pairwise linkage disequilibriums for the four SNPs are presented respectively: rs689466, rs3218625 and rs20417 polymorphisms where in strong linkage disequilibrium with each other and therefore formed a haplotype block. The two most common haplotypes, AGG and GGG (in the order of rs689466, rs3218625 and rs20417), were found to account for 94.6% of the study population. Global score test showed statistically significant differences in haplotype frequency distribution and the occurrence of grade 3 or 4 hematologic and leukopenia toxicity (global $X^2 = 6.74$, df = 1, P = 0.009, and Global $X^2 = 5.02$, df = 1, p = 0.025, respectively, Bold in Table 5). The haplotype AGG was associated with a reduced risk of grade 3 or 4 hematologic and leukopenia toxicity (P = 0.009; OR = 0.59; 95%CI = 0.39–0.88 and P = 0.025; OR = 0.61; 95%CI = 0.39–0.94, respectively), while the haplotype GGG was associated with an increased risk of grade 3 or 4 hematologic and leukopenia toxicity (P = 0.009; OR = 1.71; 95%CI = 1.14–2.56 and P = 0.025; OR = 1.65; 95%CI = 1.06–2.57, respectively). No significant association between other haplotypes and 3 or 4 toxicities was observed (Table 5).

The COX-2 SNPs and Toxicity in Patients with NSCLC

**Table 2.** Allele frequencies of the polymorphisms and the distribution of chemotherapy regimens by polymorphism group.

| Genotype | Total (%) | GP(n) | GC | NP | NC | T/P|T/P+C |
|----------|-----------|-------|----|----|----|----|------|
| rs689465 |           |       |    |    |    |    |      |
| A/A      | 178(84.0) | 115   | 12 | 22 | 13 | 6  | 10   |
| A/G      | 31(14.6)  | 18    | 5  | 3  | 3  | 1  | 1    |
| G/G      | 3(1.4)    | 0     | 0  | 1  | 0  | 0  | 2    |
| rs689466 |           |       |    |    |    |    |      |
| A/A      | 67(31.6)  | 38    | 5  | 9  | 4  | 5  | 6    |
| A/G      | 99(46.7)  | 64    | 8  | 12 | 8  | 2  | 5    |
| G/G      | 46(21.7)  | 31    | 4  | 5  | 4  | 0  | 2    |
| rs20417  |           |       |    |    |    |    |      |
| C/C      | 10(5.0)   | 1     | 0  | 0  | 0  | 0  | 0    |
| C/G      | 16(7.5)   | 11    | 1  | 2  | 0  | 1  | 1    |
| G/G      | 195(92.0) | 121   | 16 | 24 | 16 | 6  | 12   |
| rs3218625|           |       |    |    |    |    |      |
| A/A      | 0(0)      | 0     | 0  | 0  | 0  | 0  | 0    |
| A/G      | 52(24.4)  | 4     | 0  | 0  | 1  | 0  | 0    |
| G/G      | 207(97.6) | 133   | 17 | 26 | 16 | 7  | 13   |

doi:10.1371/journal.pone.0061585.t002

**Table 3.** Treated Patients with CTC Grade 3 or 4 Drug-Related Toxicities (worst grade)*.

| Toxicity                  | N (%) |
|---------------------------|-------|
| **Hematologic toxicity**  |       |
| Leukocytopenia            | 82(38.7) |
| Thrombocytopenia          | 58(27.4) |
| Anemia                    | 8(3.8) |
| **Non-hematologic toxicity** |     |
| Alopecia, any grade       | 23(10.8) |
| Cardiac toxicity          | 10(4.6) |

*Only toxicities reported in at least 3% of patients are listed.

doi:10.1371/journal.pone.0061585.t003
Table 4. Association between COX-2 polymorphisms and 3 or 4 Drug-Related Toxicities (worst grade).

| Genotype  | Total | 3 or 4 Hematologic toxicity P | 3 or 4 Leukopenia P | 3 or 4 Thrombocytopenia P | 3 or 4 Anemia P | 3 or 4 Nausea/vomiting P | Alopecia P | 3 or 4 Cardiac toxicity P |
|-----------|-------|------------------------------|--------------------|--------------------------|----------------|--------------------------|-----------|--------------------------|
| rs689465  |       |                              |                    |                          |                |                          |           |                          |
| AA        | 178   | 70                           | 53                 | 19                       | 6              | 22                       | 19        | 8                        |
| AG+GG     | 34    | 12                           | 0.711              | 0.105                    | 0.138          | 2                        | 0.516     | 6                        |
| rs689466  |       |                              |                    |                          |                |                          |           |                          |
| AA        | 67    | 17                           | 10                 | 8                        | 1              | 9                        | 6         | 3                        |
| AG        | 99    | 43                           | 33                 | 11                       | 5              | 15                       | 11        | 4                        |
| GG        | 46    | 22                           | 7                  | 7                        | 2              | 4                        | 6         | 3                        |
| AG+GG     | 145   | 65                           | 0.008*             | 0.010*                   | 0.992          | 0.206                    | 0.626     | 0.111                    |
| rs20417   |       |                              |                    |                          |                |                          |           |                          |
| GG        | 195   | 76                           | 54                 | 24                       | 7              | 24                       | 21        | 9                        |
| CG+CC     | 17    | 6                            | 0.655              | 0.770                    | 0.752          | 0.886                    | 0.255     | 0.859                    |
| rs3218625 |       |                              |                    |                          |                |                          |           |                          |
| GG        | 207   | 80                           | 56                 | 26                       | 7              | 28                       | 22        | 9                        |
| AG+AA     | 5     | 2                            | 0.811              | 0.524                    | 1.000          | 0.045*                   | 0         | 1.000                    |

*Data were calculated by unconditional logistic regression. P value shown in the table was original P value.
*Bold, P-value was still significant after the Bonferroni correction, adjusted P value were 0.024 and 0.030, respectively. + Bold, no more significance after the Bonferroni correction, adjusted P value was 0.135.

doi:10.1371/journal.pone.0061585.t004
differentiation, malignant transformation and survival by targeting a variety of genes [34]. Studies show that the directive differentiation of erythroid, myeloid, megakaryocytic progenitors is related to the level of the expression of C-MYB [35–37]. Therefore, it is suggested that the sequence variation that creates the c-MYB binding site, such as COX-2 rs689466 polymorphism, may alter the level and specificity of gene transcription. We supposed that the association of COX-2 genotypes with increased risk of grade 3 or 4 hematologic and leukopenia toxicity might be attributed to gain of function of the gene resulting from the promoter SNPs. Another reasonable explanation is that rs689466 AA homozygous increases the expression of COX-2 mRNA and enhances the transcriptional activity of COX-2, thus causing reduction of apoptosis and further reduces the risk of severe toxicity, which has been confirmed by another study [6]. While the etiology and the mechanism of these outcomes are unknown, further experiments still need to reveal the detailed molecular mechanisms.

Table 5. COX-2 haplotypes and 3 or 4 Drug-Related Toxicities (worst grade).

| Haplotypes* | A G C | p | A G G | p | G A G | p | G G C | p | G G G | p | Global score test |
|-------------|-------|---|-------|---|-------|---|-------|---|-------|---|-------------------|
| Hematologic toxicity |       |   |       |   |       |   |       |   |       |   |                   |
| 3, 4 grade, n (%) | 6(3.6) | NA | 71(43.3) | 0.009 | 2(1.2) | NA | 0(0.0) | NA | 85(51.8) | 0.009 | Global $X^2 = 6.74$, df = 1, p = 0.009 |
| 0, 1, 2 grade, n (%) | 12(4.6) | 144(55.4) | 3(1.2) | 0(0.0) | 101(38.8) |   |       |   |       |   |                   |
| Leukopenia |       |   |       |   |       |   |       |   |       |   |                   |
| 3, 4 grade | 4(3.4) | NA | 49(42.3) | 0.025 | 2(1.7) | NA | 0(0.0) | NA | 61(52.6) | 0.025 | Global $X^2 = 5.02$, df = 1, p = 0.025 |
| 0, 1, 2 grade | 14(4.5) | 166(53.9) | 3(1.0) | 0(0.0) | 125(40.6) |   |       |   |       |   |                   |
| Thrombocytopenia |       |   |       |   |       |   |       |   |       |   |                   |
| 3, 4 grade | 0(0.0) | NA | 27(51.9) | 0.981 | 0(0.0) | NA | 2(3.8) | NA | 23(44.3) | 0.981 | Global $X^2 = 0.001$, df = 1, p = 0.981 |
| 0, 1, 2 grade | 16(4.3) | 190(51.1) | 5(1.3) | 0(0.0) | 161(43.3) |   |       |   |       |   |                   |
| Anemia |       |   |       |   |       |   |       |   |       |   |                   |
| 3, 4 grade | 1(6.2) | 0.372 | 6(37.5) | 0.281 | 1(6.2) | 0.056 | 0(0.0) | NA | 8(50.0) | 0.614 | Global $X^2 = 4.30$, df = 3, p = 0.212 |
| 0, 1, 2 grade | 17(4.1) | 209(51.2) | 4(1.0) | 0(0.0) | 178(43.6) |   |       |   |       |   |                   |
| Nausea/vomiting |       |   |       |   |       |   |       |   |       |   |                   |
| 3, 4 grade | 4(7.1) | 0.259 | 28(50.0) | 0.834 | 0(0.0) | NA | 0(0.0) | NA | 24(42.8) | 0.803 | Global $X^2 = 1.28$, df = 2, p = 0.528 |
| 0, 1, 2 grade | 14(3.8) | 187(50.8) | 5(1.4) | 0(0.0) | 162(44.0) |   |       |   |       |   |                   |
| Alopecia |       |   |       |   |       |   |       |   |       |   |                   |
| 3, 4 grade | 0(0.0) | NA | 23(50.0) | 0.925 | 1(2.2) | NA | 2(4.3) | NA | 20(43.5) | 0.925 | Global $X^2 = 0.009$, df = 1, p = 0.925 |
| 0, 1, 2 grade | 16(4.2) | 194(51.3) | 4(1.1) | 0(0.0) | 164(43.4) |   |       |   |       |   |                   |
| Cardiac toxicity |       |   |       |   |       |   |       |   |       |   |                   |
| 3, 4 grade | 0.5(2.5) | NA | 9.5(47.5) | 0.736 | 0(0.0) | NA | 0.5(2.5) | NA | 9.5(47.5) | 0.736 | Global $X^2 = 0.14$, df = 2, p = 0.736 |
| 0, 1, 2 grade | 17(4.2) | 206(51.0) | 5(1.2) | 0(0.0) | 176(43.5) |   |       |   |       |   |                   |

Order of polymorphisms: rs689466, rs3218625, rs20417. *Haplotypes were omitted if the estimated haplotype probability was less than 5%; Bold, P-value was significant. NA, not applicable. doi:10.1371/journal.pone.0061585.t005

The COX-2 SNPs and Toxicity in Patients with NSCLC

Therefore, it is suggested that the sequence variation that creates the c-MYB binding site, such as COX-2 rs689466 polymorphism, may alter the level and specificity of gene transcription. We supposed that the association of COX-2 genotypes with increased risk of grade 3 or 4 hematologic and leukopenia toxicity might be attributed to gain of function of the gene resulting from the promoter SNPs. Another reasonable explanation is that rs689466 AA homozygous increases the expression of COX-2 mRNA and enhances the transcriptional activity of COX-2, thus causing reduction of apoptosis and further reduces the risk of severe toxicity, which has been confirmed by another study [6]. While the etiology and the mechanism of these outcomes are unknown, further experiments still need to reveal the detailed molecular mechanisms.

Previous clinical studies have suggested the expression of COX-2 as a predictive factor for survival in NSCLC patients [15,38]. In the study by Edelman et al, patients with advanced NSCLC expressing moderate to high COX-2 protein levels had worse survival than those with lower expression levels [38]. Groen et al. reported that the high expression of COX-2 with better PS and adenocarcinoma was associated with better survival [39]. Few studies have been reported thus far regarding the association between COX-2 rs689466 polymorphism and clinical outcomes in NSCLC patients. Nan Bi et al. examined five functional COX-2 polymorphisms and suggested that only COX-2 rs689466 polymorphism was a potential predictive marker for survival in locally advanced NSCLC patients treated with chemoradiotherapy or radiotherapy alone and the GA and GG genotypes were significantly correlated with better overall survival and with longer progression-free survival compared with the rs689466 AA genotype [20]. In addition, a recent study that included 231 patients with lung cancer also suggested that COX-2 rs689466 may be a risk factor for the development of lung cancer [40].

All the studies presented above, including ours, suggest that COX-2 rs689466 polymorphism plays an important role in cancer development and might be a useful molecular indicator of cancer risk, prognosis, response to treatment and toxicity.

However, our study has several limitations. First, treatment heterogeneity, i.e. different combination regimens and the duration of the therapy may influence the result. However, all
the ORs and 95% CIs had been adjusted by types of treatment regimens, including cisplatin versus carboplatin, taxanes versus non-taxanes and the number of cycles of chemotherapy received during first-line treatment in logistic regression model, thus the potential confounding factors were minimized. Second, in our study, we found that COX-2 rs689466 variant A allele frequency was similar to those that has been published in Chinese population [14,20], but this SNP is ethnic specific compared with Caucasian [21,40], thus our results should be validated among different ethnic populations. Finally, prior studies failed to show any significant association between COX-2 expression and toxicity. After reviewing these articles carefully, we consider that they mainly focused on the role of COX-2 in cancer development, epidemiology and predictive role for survival [12,13,15,38,39]. Instead, our study chose to only focus on drug-related toxicity, mainly hematologic toxicity, and the association between toxicity and COX-2 polymorphisms, not COX-2 expression. Further prospective validation studies should be carried out to replicate the findings.

Our study also has several strengths. We assess the grading of toxicities according to the National Cancer Institute Common Toxicity Criteria version 3.0 among clinicians comprehensively. A relatively large number of patients with advanced NSCLC receiving platinum-based chemotherapy were carried out independently without the knowledge of polymorphism status enrolled in this study. All patients were treated at the same hospital. It seems that our finding is not likely to have been obtained by chance.

To the best of our knowledge, this is the first study showing that the COX-2 polymorphism could predict toxicity outcomes in patients with advanced stage NSCLC treated with platinum-based chemotherapy. We found that the rs689466 polymorphism was associated with severe hematologic and leukopenia toxicity risk.

Conclusion

This investigation for the first time suggested that polymorphism in COX-2 rs689466 may be a potent bio-marker in predicting severe hematologic toxicity in NSCLC patients after platinum-based chemotherapy.

Acknowledgments

We thank our volunteers for donating their blood and collaborators for collection of blood sample and information.

Author Contributions

Conceived and designed the experiments: CZ. Performed the experiments: FZ XL. Analyzed the data: FZ GG SR. Contributed reagents/materials/analysis tools: FZ GG SR YH. Wrote the paper: FZ GG SR.

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. Jemal A, Siegel R, Xu J, Ward E (2010) Cancer statistics, 2010. CA Cancer J Clin 60:277–300.
3. Schiller JH, Harrington D, Belani CP, Sandler A, Gierga D, et al. (2002) Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 346: 92–98.
4. Scaglotti GV, Parikh P, von Pawel J, Biesma B, Vansteenkiste J, et al. (2008) Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. J Clin Oncol 26:3453–51.
5. Rabik CA, Dolan ME (2007) Molecular mechanisms of resistance and toxicity associated with platinum-based agents. Cancer Treat Rev 33:9–23.
6. Gu S, Wu Q, Zhao X, Wu W, Gao Z, et al. (2012) Association of CASP8 polymorphism with hematologic toxicity in patients with advanced non-small-cell lung carcinoma treated with platinum-based chemotherapy. Cancer Sci 103:1451–9.
7. Bosdaks T, Pantos A, Belis E, Petros C (2007) Designing platinum compounds in cancer structures and mechanisms. Cancer Therapy 5: 537–543.
8. Zimmermann KC, Sarbia M, Weber AA, Borchard F, Gabbett HE, et al. (1999) Cyclooxygenase-2 expression in human esophageal carcinoma. Cancer Res 59:198–204.
9. Setia S, Vaish V, Sanyal SN (2012) Chemopreventive effects of NSAIDs as inhibitors of cyclooxygenase-2 and inducers of apoptosis in experimental lung carcinogenesis. Mol Cell Biochem 366:89–99.
10. Sun Y, Tang XM, Half E, Kuo MT, Sincrope FA (2002) Cyclooxygenase-2 overexpression reduces apoptotic susceptibility by inhibiting the cytochrome c-dependent apoptotic pathway in human colon cancer cells. Cancer Res 62:6323–8.
11. Cory S, Adams JM (2002) The Bcl2 family: regulators of the cellular life or death switch. Nat Rev Cancer 2:647–56.
12. Ochiai M, Oguri T, Isobe T, Ishioka S, Yamakido M (1999) Cyclooxygenase-2 (COX-2) mRNA expression levels in normal lung tissues and non-small cell lung cancers. Jpn J Cancer Res 90:1338–43.
13. Wolff H, Saukkonen K, Anttila S, Karjalainen A, Vainio H, et al. (1998) COX-2 expression in human lung adenocarcinoma in Chinese population. PLoS One 6:e21894.
14. Kim SH, Park HS (2006) Genetic markers for differentiating aspirin-hypersensitivity. Yonsei Med J 47:15–21.
15. Zhao D, Xu D, Zhang X, Wang L, Tan W, et al. (2009) Interaction of cyclooxygenase-2 variants and smoking in pancreatic cancer: a possible role of nucleophosmin. Gastroenterology 136:1659–68.
16. Bi N, Yang M, Zhang L, Chen X, Ji W, et al. (2010) Cyclooxygenase-2 genetic variants are associated with survival in unscreened locally advanced non-small cell lung cancer. Clin Cancer Res 16:2383–90.
17. Hoff JH, te Morsche RH, Roels FH, van der Logt EM, Nagengast FM, et al. (2009) COX-2 polymorphisms –765G>C and –1195A>G and colorectal cancer risk. World J Gastroenterol 15: 4641–4563.
18. Iglesias D, Nejla N, Azouit MM, Schwartz S J, Gonzalez-Aguilera JJ, et al. (2007) Effect of COX2 –765G>C and c.3618A>G polymorphisms on the risk and survival of sporadic colorectal cancer. Cancer Causes Control 20:1421–1429.
19. Papaprilis A, Hill MR, Broll DJ, McAmudy RJ, Marshall RP, et al. (2002) Common promoter variant in cyclooxygenase-2 represses gene expression: evidence of role in acute-phase inflammatory response. Arterioscler Thromb Vasc Biol 22:1631–1636.
20. Zhang XM, Zhong R, Liu L, Wang Y, Yuan JX, et al. (2011) Smoking and COX-2 functional polymorphisms interact to increase the risk of gastric cardia adenocarcinoma in Chinese population. PLoS One 6:e21894.
21. Ozer H, Armitage JO, Bennett CL, Crawford JF, Demetri GD, et al. (2000) 2000 update of recommendations for the use of hematopoietic colony-stimulating factors: Evidence-based clinical practice guidelines. J Clin Oncol 18:3538–3585.
22. Zhang L, Gao G, Li X, Ren S, Li A, et al. (2012) Association between single nucleotide polymorphisms (SNPs) and toxicity of advanced non-small-cell lung cancer patients treated with chemotherapy. PLoS One 7: e40350.
23. Shi YY, He L (2009) SHEs, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 15:97–8.
24. Li Z, Zhang Z, He Z, Tang W, Li T, et al. (2009) A partition-higation- combination-subdivision EM algorithm for haplotype inference with multi-allelic markers: update of the SHEs algorithm. Yonsei Med J 19:519–23.
25. Sonis ST, O’Donnell KE, Popat R, Bragdon C, Phelan S, et al. (2004) The relationship between mucosal cyclooxygenase-2 (COX-2) expression and experimental radiation-induced mucositis. Oral Oncol 40:170–176.
26. Keshuk M, Gomsen E, Kilic M, Genrucker S, Can B, et al. (2006) Increased expression of cyclooxygenase-2 (COX-2) in radiation-induced small bowel Injury in rats. J Surg Res 135:76–84.
27. Kishi K, Petersen S, Petersen C, Hunter N, Mason K, et al. (2000) Preferential targeting of cancer cells. Cancer Res 60:1326–31.
32. Stone HB, Coleman CN, Anscher MS, McBride WH (2003) Effects of radiation on normal tissue: consequences and mechanisms. The Lancet Oncology 4:529–36.
33. Dixon DA (2003) Regulation of COX-2 expression in human cancers. Prog Exp Tumor Res 37:52–71.
34. Ramsay RG, Barton AL, Gonda TJ (2003) Targeting c-MYB expression in human disease. Expert Opin Ther Targets 7:215–248.
35. Soza-Ried C, Hess I, Netuschil N, Schorpp M, Boehm T (2010) Essential role of c-myb in definitive hematopoiesis is evolutionarily conserved. Proc Natl Acad Sci U S A 107:17304–8.
36. Greig KT, Carotta S, Nutt SL (2006) Critical roles for c-Myb in hematopoietic progenitor cells. Semin Immunol 20:247–56.
37. Vegiopoulos A, García P, Emambokus N, Frampton J (2006) Coordination of erythropoiesis by the transcription factor c-Myb. Blood 107:4703–10.
38. Edelman MJ, Watson D, Wang X, Morrison G, Kratzke RA, et al. (2008) Eicosanoid modulation in advanced lung cancer: Cyclooxygenase-2 expression is a positive predictive factor for celecoxib+chemotherapy—Cancer and Leukemia Group B Trial 30203. J Clin Oncol 26:848–855.
39. Groen HJM, Sietsma H, Vincent A, Hochstenbag MMH, van Putten JWG, et al. (2011) Randomized, placebo-controlled phase III study of docetaxel plus carboplatin with celecoxib and cyclooxygenase-2 expression as a biomarker for patients with advanced non-small-cell lung cancer: The NVALT-4 study. J Clin Oncol 29:4320–4326.
40. Coskunpinar E, Eraltan IY, Turna A, Agachan B (2011) Cyclooxygenase-2 gene and lung carcinoma risk. Med Oncol 28:1436–1440.