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Original Article

Genetic polymorphisms in oxidative stress-related genes are associated with clinical outcome in patients with advanced non-small cell lung cancer receiving tyrosine kinase inhibitors

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Abstract: Many types of cancer have high antioxidant capacity that effectively scavenges reactive oxygen species and thus protect cancer cells against oxidative damage. The aim of this study was to examine the effect of 20 single nucleotide polymorphisms (SNPs) in 20 oxidative stress-related genes on clinical outcome in 219 patients with advanced non-small cell lung cancer (NSCLC) who were treated with EGFR tyrosine kinase inhibitors (TKIs). We assessed the associations of SNPs with prognosis in all patients as well as stratified by clinical characteristics. Three SNP (rs1695, rs2333227 and rs699512) were significantly associated with overall survival (OS). In a multivariate analysis, rs1695 AA and rs2333227 AG/GG genotypes were identified as independent prognostic factors for poor OS. Stratification analyses revealed that these 3 SNPs remained significantly associated with OS. Furthermore, there was a strong gene-dosage effect of these 3 SNPs on OS that patients with increasing number of unfavorable genotypes had significantly increased death risk. In conclusion, our findings provide the first evidence that genetic variants in oxidative stress-related genes may influence treatment outcome in advanced NSCLC patients receiving EGFR TKIs.

Keywords: Tyrosine kinase inhibitor, lung cancer, glutathione S-transferases P1, myeloperoxidase, biliverdin reductase A, oxidative stress, single nucleotide polymorphism

Introduction

Lung cancer is the leading cause of cancer-related death, accounting for 19.59% of the total cases and 24.87% of the deaths in 2010 in China [1]. Approximately 85% of lung cancers are non-small cell lung cancer (NSCLC) [2]. An activating mutation of epidermal growth factor receptor (EGFR) is present in 40.9% of NSCLC [3]. These mutations are most commonly found in lung adenocarcinomas from East Asian non-smokers. Dysregulated tyrosine kinase activity of EGFR, caused by mutations, result in aberrant EGFR signaling and promote EGFR-mediated pro-survival and anti-apoptotic signals through pathways including RAS/RAF/ERK, PI3K/Akt, and STAT pathways [4]. EGFR mutations predict better response to EGFR tyrosine kinase inhibitors (TKIs) compared with standard platinum-based chemotherapy in patients with advanced NSCLC [5, 6].

Reactive oxygen species (ROS) are now appreciated to act as signaling molecules involved in the regulation of various physiological processes, and therefore are essential for maintaining normal cell function. However, overproduction and cumulative production of ROS can cause damage to DNA, proteins, lipids, and other macromolecules, and induce cell death. Accordingly,

oxidative stress and oxidative damage have been implicated in the pathogenesis and progression of many human diseases, including cancer [7]. Although the exact mechanism by which ROS promote tumorigenesis remains poorly understood, DNA damage appears to play a key role in cancer development. Cancer cells exhibit greater oxidative stress, which in turn promote cell proliferation, thus contributing to cancer progression [7, 8]. In addition, elevated ROS levels in cancer cells lead to enhanced activation of the antioxidant defense system and the upregulation of pro-survival molecules, which promote cell survival upon oxidative stress [7, 8]. Reduced intracellular ROS levels through administration of antioxidants impair cell proliferation and survival in some types of cancer, including colorectal cancer (CRC) [9], glioma [10] and lymphomas [11]. Redox pathways may be potential targets for cancer therapy.

Anticancer agents kill cancer cells partly through increased formation of ROS [12], whereas anticancer drug-resistant cancer cells exhibit higher levels of antioxidant enzymes such as glutathione peroxidase 1 (GPX1), and adapt to survive at high ROS levels [13]. For example, the relative effectiveness of paclitaxel is inversely correlated with antioxidant capacity of cancer cells [14, 15]. Single nucleotide polymorphisms (SNPs) leading to changes in expression and function of antioxidant enzymes may affect the effectiveness of anticancer drugs. Recent studies found that enhanced antioxidant capacity was involved in mediating EGFR TKI-resistance [16-18]. Therefore, SNPs in antioxidant genes may be associated with survival outcome in cancer patients receiving TKI therapy. The aim of this study was to investigate the effect of 20 SNPs in 20 oxidative stress-related gene on the prognosis of patients with advanced NSCLC who were treated with EGFR TKIs.

Materials and methods

Patients

A total of 219 patients with advanced-stage NSCLC were recruited from four hospitals between 2010 and 2014. All patients met the following inclusion criteria: histologically or cytologically proven NSCLC; TNM stage IIIB and IV; Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0, 1 or 2; aged > 18 years; normal liver and renal functions; normal results of completed blood counts and urinalysis. Patients received a standard oral daily dose of 250 mg gefitinib or 150 mg erlotinib until either intolerable toxicity or disease progression occurred. The smoking status was categorized as never, current and former smokers as previously described [19]. All patients gave written informed consent. Three ml of peripheral blood were collected from each patient, according to the protocol approved by the Ethics Committees of Shanghai Chest Hospital, Quanzhou First Hospital, Taizhou Central Hospital, and Taizhou Hospital.

Genotyping

SNPs in oxidative stress-related genes were selected based on the published population-based genetic associations. It is reasonable to assume that SNPs associated with cancer susceptibility, prognosis and treatment outcomes are potentially functional in response to anticancer drugs commonly shared in different types of cancer. For statistical power consideration, only SNPs with a minor allele frequency (MAF) > 0.05 in the populations of Chinese Han were selected for genotyping. As a result, a total of 20 candidate SNPs were selected from 20 oxidative stress-related genes for genotyping (Table S1).

Genomic DNA was extracted from the peripheral leukocytes using TaKaRa MiniBEST Whole Blood Genomic DNA Extraction Kit (TakaRa, Dalian, China) according to manufacturer's instructions. All SNP were genotyped using a PCR-ligation detection reaction (LDR) method as previously described [19]. In order to validate the genotyping accuracy, 10% of the samples were randomly selected for repeated genotyping by both PCR-LDR and direct sequencing, and the results showed 100% concordant.

Statistical analyses

All statistical analyses were performed using SPSS 19.0 software package (SPSS Inc, Chicago, USA) with a two-sided test. The progression-free survival (PFS) and overall survival (OS) rates were calculated using the Kaplan–Meier method, and the log-rank test was used to compare different survival curves. Univariate and multivariable Cox proportional hazard mod-

Table 1. Clinicopathological characteristics of patients

Characteristics	No.	%
Age, years	57 ± 10.3	
Sex		
Male	107	48.9
Female	112	51.1
ECOG PS		
0-1	207	94.5
2	11	5
TNM		
IIIB	56	25.6
IV	163	74.4
Smoking status		
Never	151	68.9
Current	47	21.5
Former	17	7.8
Unkown	4	1.8
Histology		
Adenocarcinoma	194	88.6
Other	25	11.4

els were used to calculate the crude and adjusted hazard ratios (HRs) and their 95% confidence intervals (CIs), respectively. A P value < 0.05 was considered statistically significant.

Results

Patient characteristics

The characteristics of the 219 patients with advanced NSCLC were summarized in **Table 1**. The mean patient age was 57 years for women and 58 years for men. The median follow-up of patients was 19.0 (95% CI 17.1-20.9) months. One hundred sixty two (74.0%) patients died during the follow-up period.

Association of SNPs with PDF and OS in NSCLC patients

Two SNPs (rs1801282 and rs1937840) deviated significantly from Hardy-Weinberg equilibrium (P < 0.05, Table S2) and were excluded from further analysis. The associations of oxidative stress-related genetic factors with DFS and OS of patients with advanced NSCLC were presented in Table S2. No SNP showed association with PFS under any genetic model (Table S2). However, we found significant associations of glutathione S-transferases P1 (GSTP1) rs1695, myeloperoxidase (MPO) rs2333227 and biliverdin reductase A (BLVRA) rs699512 with OS. In multivariate analysis, including age, sex, TNM stage, ECOG PS, histology, and smoking status, rs1695 AA genotype (AA vs AG+GG, adjusted HR = 1.458, 95% CI 1.033-2.059, P = 0.032) and rs2333227 AA (AG vs GG, adjusted HR = 1.452, 95% CI 1.014-2.079, P = 0.042) and AG (AA vs GG, adjusted HR = 2.829, 95% CI 1.211-6.607, P = 0.016) genotypes correlated with worse survival and were all identified as independent prognostic factors for poor OS (**Table 2**, **Figure 1**). Although rs699512 G allele was associated with poor OS (AG *vs* AA, HR = 1.452, 95% CI 1.045-2.018, P = 0.026; AG+GG *vs* AA, HR = 1.450, 95% CI 1.059-1.986, P = 0.021) compared with AA genotype, the difference disappeared after adjustment for age, sex, TNM stage, ECOG PS, histology, smoking status (P > 0.05). We further examined the cumulative effect of rs1695, rs2333227 and rs699512 on OS. The number of unfavorable genotype (rs1695 AA genotype, rs2333227 AA and AG genotypes, and rs699512 AG and GG genotypes) was positively correlated with the risk of mortality. Compared with zero unfavorable genotypes, the effect of 1, 2 and 3 unfavorable genotypes was as follows: adjusted HR = 1.852, 95% CI 1.066-3.218, P = 0.029; adjusted HR = 2.528, 95% CI 1.454-4.396, P = 0.001; adjusted HR = 2.745, 95% CI 1.419-5.309, P = 0.003, respectively (P for trend = 0.001; **Figure 1D**).

Stratified analyses of rs1695, rs2333227 and rs699512 on OS in NSCLC patients

The associations between rs1695, rs2333227 and rs699512, and OS were further investigated by stratification of age, sex, TNM stage, histology and smoking status. As shown in **Table 3**, there was a significant toward to a high risk of mortality associated with rs1695 AA genotypes in the following subjects: subjects aged ≤ 60 years; those with TNM stage IV; those with adenocarcinoma; smokers; and males (**Table 3**). For rs2333227, an increased risk of mortality was observed in the following subjects: subjects aged ≤ 60 years; those with TNM stage IV; those with adenocarcinoma; and smokers. For rs699512, an increased risk of mortality was observed in the following subjects: females; never-smokers; and subjects with adenocarcinoma.

Table 2. Associations between rs1695, rs2333227 and rs699512 and OS

SNP	Genotype	Events/patients	Univariate		Multivariate	
			HR (95% CI)	*P* value	HR (95% CI)[a]	*P* value
rs1695						
	AA	106/136	1		1	
	AG	52/74	0.719 (0.514-1.005)	0.053	0.729 (0.513-1.035)	0.077
	GG	4/9	0.361 (0.133-0.982)	0.046	0.367 (0.131-1.023)	0.055
	AG+GG	56/83	1		1	
	AA	106/136	1.488 (1.072-2.064)	0.017	1.458 (1.033-2.059)	0.032
rs2333227						
	GG	110/157	1		1	
	AG	45/55	1.424 (1.006-2.016)	0.046	1.452 (1.014-2.079)	0.042
	AA	7/7	2.273 (1.056-4.896)	0.036	2.829 (1.211-6.607)	0.016
	GG	110/157	1		1	
	AA+AG	52/62	1.499 (1.076-2.086)	0.017	1.543 (1.093-2.176)	0.014
rs699512						
	AA	66/100	1		1	
	AG	78/96	1.452 (1.045-2.018)	0.026	1.392 (0.984-1.970)	0.062
	GG	18/23	1.441 (0.855-2.428)	0.170	1.320 (0.772-2.257)	0.310
	AA	66/100	1		1	
	AG+GG	96/119	1.450 (1.059-1.986)	0.021	1.377 (0.989-1.918)	0.058
risk genotypes[b]						
	0	18/33	1		1	
	1	58/82	1.853 (1.091-3.148)	0.022	1.852 (1.066-3.218)	0.029
	2	62/77	2.439 (1.436-4.141)	0.001	2.528 (1.454-4.396)	0.001
	3	24/27	2.773 (1.499-5.128)	0.001	2.745 (1.419-5.309)	0.003

[a]Adjusted for age, sex, ECOG PS, TNM stage, smoking status and histology. [b]Risk genotype: rs1695 AA, rs2243828 AG/GG, rs699512 AG/GG.

Discussion

Despite the initial beneficial effect of EGFR TKIs in NSCLC patients with EGFR activating mutations, most patients eventually develop acquired resistance to EGFR-TKIs. Therefore, improving treatment outcomes for this group of NSCLC patients remains an area of high unmet clinical need. Although somatic EGFR mutations are the major determinants of response to EGFR TKI therapy, recent studies have found that genetic variants affect the clinical outcome of NSCLC patients treated with EGFR TKIs [20-22]. Increased antioxidant capacity is mechanisms that has been widely implicated in anticancer drug resistance [15-17, 23]. In the present study, we found that rs1695, rs2333227 and rs699512 were associated with poor OS in advanced NSCLC patients who received EGFR TKIs. To the best of our knowledge, this is the first study evaluating the impact of genetic polymorphisms in oxidative stress-related genes on treatment outcome in advanced NSCLC patients.

The oxidative stress-related pathways have been widely studied for their roles in cancer development and treatment. For example, ROS is shown to mediate EGFR TKI-resistance through maintenance of EGFR tyrosine phosphorylation, whereas inhibiting ROS production restored gefitinib sensitivity in resistant breast cancer cells [18]. Increased glutamine metabolism was observed in erlotinib-resistant lung cancer cell lines [16, 17]. Glutathione S-transferases play important role in detoxification through the conjugation of glutathione to electrophilic xenobiotics. GSTP1 isoenzyme is upregulated in various types of cancer, such as NSCLC [24-26] and CRC [27], and might influence response to platinum-based chemotherapy [28]. GSTP1 rs1695, leading to the substitution of isoleucine to valine at 105 amino acid position (Ile105Val), exhibits a significant influ-

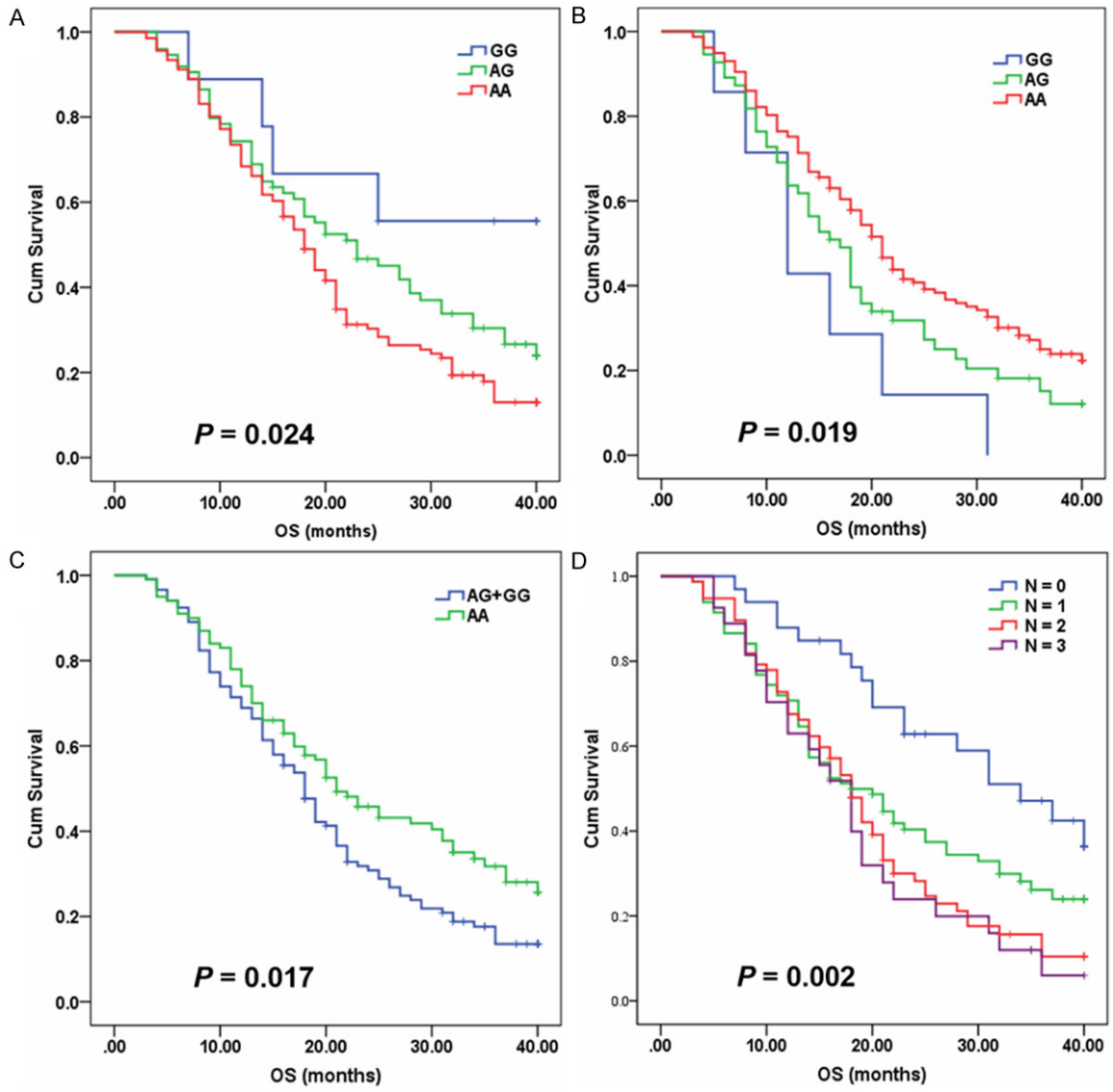

Figure 1. Kaplan-Meier curves of OS for advanced NSCLC patients treated with EGFR TKIs according to genotypes. A. rs1695. B. rs2333227. C. rs699512. D. Cumulative effect of unfavorable genotypes at rs1695, rs2333227 and rs699512 on OS.

ence on enzymatic activity [29], and is related to clinical outcome of patients receiving chemotherapy [30-32]. In a cohort of 115 advanced NSCLC patients treated with platinum-based chemotherapy, those with rs1695 G allele had a higher response rate and better prognosis [31]. Jun et al. reported the association of rs1695 GG genotype with better clinical outcome in advanced CRC patients who were treated with 5-FU-oxaliplatin-based chemotherapy [30]. These results are in agreement with our findings that G allele was associated with better survival time. However, there are inconsistent associations between rs1695 and prognosis of cancer patients. For example, Liu et al. [32] reported that rs1695 GG genotype was linked to worse prognosis in osteosarcoma patients treated with doxorubicin based chemotherapy. Genetic heterogeneity may partly explain these inconsistent results despite different type of cancer and therapeutic regimen. Furthermore, the frequencies of the G allele and GG genotype of rs1695 were remarkable higher, and rs1695 deviated significantly from Hardy-Weinberg equilibrium in Liu study [32].

MPO is an endogenous oxidant lysosomal enzyme secreted by neutrophils that catalyze

Table 3. Stratification analysis of rs1695, rs2333227 and rs699512 associated with survival of NSCLC patients

Variables[a]	rs1695[b]		rs2333227[b]		rs699512[b]	
	HR (95% CI)[c]	*P* value	HR (95% CI)[c]	*P* value	HR (95% CI)[c]	*P* value
Age (years)						
≤ 60	1.665 (1.047-2.648)	0.031	1.722 (1.081-2.742)	0.022	1.276 (0.810-2.011)	0.293
> 60	1.338 (0.773-2.316)	0.298	1.693 (0.977-2.933)	0.061	1.622 (0.962-2.734)	0.069
Sex						
Male	4.622 (2.206-9.685)	< 0.001	1.654 (0.996-2.745)	0.052	0.946 (0.594-1.509)	0.817
Female	0.779 (0.497-1.221)	0.276	1.691 (1.026-2.785)	0.039	1.945 (1.224-3.090)	0.005
TNM						
IIIB	1.272 (0.557-2.908)	0.568	1.140 (0.558-2.332)	0.719	1.887 (0.904-3.938)	0.091
IV	1.521 (1.034-2.239)	0.033	1.918 (1.279-2.876)	0.002	1.210 (0.824-1.777)	0.332
Smoking status						
Never-smoker	0.980 (0.663-1.450)	0.920	1.421 (0.937-2.154)	0.098	1.521 (1.026-2.255)	0.037
Smoker	6.527 (2.357-18.076)	< 0.001	2.193 (1.156-4.161)	0.016	0.865 (0.452-1.656)	0.662
Histology (%)						
Adenocarcinoma	1.692 (1.162-1.464)	0.006	1.796 (1.229-2.624)	0.003	1.487 (1.033-2.142)	0.033
Others	0.601 (0.148-2.437)	0.476	1.422 (0.418-4.836)	0.573	0.832 (0.278-2.489)	0.742

[a]ECOG PS was excluded for stratification analysis due to only 11 cases of NSCLC patients with ECOG PS 2. [b]Rs1695: AA vs AG+GG; rs2333227: AA+AG vs GG; rs699512: AG+GG vs AA. [c]Adjusted for age, sex, ECOG PS, TNM stage, smoking status and histology, as appropriate.

formation of numerous reactive oxidant species. Rymaszewski et al. [33] reported that administration of MPO inhibitor reduced butylated hydroxytoluene-promotion of MCA-induced lung carcinogenesis by 50% in BALB mice. MPO may play an important role in inflammation promoted lung carcinogenesis. Rs2333227 is located in the strong stimulatory protein 1 (SP1) transcription factors binding site in the promoter region of the MPO gene, and thus affects MPO expression [34]. The A allele is associated with reduced MPO mRNA and protein levels attributable to the disruption of a SP1-binding site. Many studies have shown that rs2333227 is associated with risk of many human diseases, including lung cancer [35]. In this study, we found that rs2333227 A allele conferred a high risk of death for NSCLC patients. In a more recent study, rs2243828, located in the promoter region of the MPO gene, was reported as a prognostic marker for patients with aggressive B-cell non-Hodgkin lymphoma [36]. Rs2243828 is 122 bp away from rs2333227, and rs2243828 G allele is in linkage disequilibrium with rs2333227 A allele. Therefore, rs2243828 G allele may be linked to lower MPO expression. Together with these findings, decreased MPO expression may be beneficial for cancer cells to survive under anticancer treatment. However, the lung cancer graft is slower growing in an MPO-knockout mouse, indicating that a non-enzymatic function of MPO is required for cancer growth in the later phases of tumor progression [33]. These imply that cancer cell is more susceptible to oxidative stress under extreme conditions. Further studies are warranted to elucidate the role of MPO in NSCLC.

BLVRA plays an important role in the maintenance of intracellular redox homeostasis through its production of bilirubin, a major natural and potent antioxidant [37, 38]. Recent data show that BLVRA can act as a dual-specificity protein kinase and transcription factor, involved in various cellular functions [38]. BLVRA is upregulated in chemoresistant cancer cell lines, and inhibition of BLVRA reverses drug resistance in cancer cells [39, 40]. Meanwhile, the present study found that the BLVRA rs699512 G allele, resulting in the substitution of threonine to alanine at 3 amino acid position (Thr3Ala), was significantly associated with worse survival in univariate Cox analysis. In a previous association study for patients with essential hypertension, rs699512 G allele was related to reduced risk of hypertension, and G allele carriers had lower systolic and diastolic blood pressures [41]. Rs699512 is possible functional SNP that could impair BLVRA function.

In conclusion, the current findings provide evidence that genetic variants in oxidative stress-related genes may modify prognosis in advanced NSCLC patients treated with EGFR TKIs. Cellular redox state is associated with the treatment efficacy of EGFR TKIs in NSCLC patients with activating EGFR mutations. Further prospective studies with large NSCLC populations receiving EGFR TKIs are required to confirm the predictive value of SNPs in oxidative stress-related genes for treatment outcome.

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Disclosure of conflict of interest

None.

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References

[1] Chen W, Zheng R, Zhang S, Zhao P, Zeng H, Zou X and He J. Annual report on status of cancer in China, 2010. Chin J Cancer Res 2014; 26: 48-58.

[2] Ramalingam S and Belani C. Systemic chemotherapy for advanced non-small cell lung cancer: recent advances and future directions. Oncologist 2008; 13 Suppl 1: 5-13.

[3] Kimura H, Ohira T, Uchida O, Matsubayashi J, Shimizu S, Nagao T, Ikeda N and Nishio K. Analytical performance of the cobas EGFR mutation assay for Japanese non-small-cell lung cancer. Lung Cancer 2014; 83: 329-333.

[4] Zhang Z, Stiegler AL, Boggon TJ, Kobayashi S and Halmos B. EGFR-mutated lung cancer: a paradigm of molecular oncology. Oncotarget 2010; 1: 497-514.

[5] Linardou H, Dahabreh IJ, Bafaloukos D, Kosmidis P and Murray S. Somatic EGFR mutations and efficacy of tyrosine kinase inhibitors in NSCLC. Nat Rev Clin Oncol 2009; 6: 352-366.

[6] Pao W and Chmielecki J. Rational, biologically based treatment of EGFR-mutant non-small-cell lung cancer. Nat Rev Cancer 2010; 10: 760-774.

[7] Holmstrom KM and Finkel T. Cellular mechanisms and physiological consequences of redox-dependent signalling. Nat Rev Mol Cell Biol 2014; 15: 411-421.

[8] Gupta SC, Hevia D, Patchva S, Park B, Koh W and Aggarwal BB. Upsides and downsides of reactive oxygen species for cancer: the roles of reactive oxygen species in tumorigenesis, prevention, and therapy. Antioxid Redox Signal 2012; 16: 1295-1322.

[9] Chinery R, Beauchamp RD, Shyr Y, Kirkland SC, Coffey RJ and Morrow JD. Antioxidants reduce cyclooxygenase-2 expression, prostaglandin production, and proliferation in colorectal cancer cells. Cancer Res 1998; 58: 2323-2327.

[10] Martin V, Herrera F, Garcia-Santos G, Antolin I, Rodriguez-Blanco J and Rodriguez C. Signaling pathways involved in antioxidant control of glioma cell proliferation. Free Radic Biol Med 2007; 42: 1715-1722.

[11] Sharma R and Vinayak M. Antioxidant alpha-tocopherol checks lymphoma promotion via regulation of expression of protein kinase C-alpha and c-Myc genes and glycolytic metabolism. Leuk Lymphoma 2012; 53: 1203-1210.

[12] Riley PA. Free radicals in biology: oxidative stress and the effects of ionizing radiation. Int J Radiat Biol 1994; 65: 27-33.

[13] Schulz R, Emmrich T, Lemmerhirt H, Leffler U, Sydow K, Hirt C, Kiefer T, Link A and Bednarski PJ. Identification of a glutathione peroxidase inhibitor that reverses resistance to anticancer drugs in human B-cell lymphoma cell lines. Bioorg Med Chem Lett 2012; 22: 6712-6715.

[14] Alexandre J, Batteux F, Nicco C, Chereau C, Laurent A, Guillevin L, Weill B and Goldwasser F. Accumulation of hydrogen peroxide is an early and crucial step for paclitaxel-induced cancer cell death both in vitro and in vivo. Int J Cancer 2006; 119: 41-48.

[15] Ramanathan B, Jan KY, Chen CH, Hour TC, Yu HJ and Pu YS. Resistance to paclitaxel is proportional to cellular total antioxidant capacity. Cancer Res 2005; 65: 8455-8460.

[16] Serizawa M, Kusuhara M, Zangiacomi V, Urakami K, Watanabe M, Takahashi T, Yamaguchi K, Yamamoto N and Koh Y. Identification of metabolic signatures associated with erlotinib resistance of non-small cell lung cancer cells. Anticancer Res 2014; 34: 2779-2787.

[17] Weaver Z, Difilippantonio S, Carretero J, Martin PL, El Meskini R, Iacovelli AJ, Gumprecht M,

Kulaga A, Guerin T, Schlomer J, Baran M, Kozlov S, McCann T, Mena S, Al-Shahrour F, Alexander D, Wong KK and Van Dyke T. Temporal molecular and biological assessment of an erlotinib-resistant lung adenocarcinoma model reveals markers of tumor progression and treatment response. Cancer Res 2012; 72: 5921-5933.

[18] Giordano CR, Mueller KL, Terlecky LJ, Krentz KA, Bollig-Fischer A, Terlecky SR and Boerner JL. A targeted enzyme approach to sensitization of tyrosine kinase inhibitor-resistant breast cancer cells. Exp Cell Res 2012; 318: 2014-2021.

[19] Zhang X, Chen Q, He C, Mao W, Zhang L, Xu X, Zhu J and Chen B. Polymorphisms on 8q24 are associated with lung cancer risk and survival in Han Chinese. PLoS One 2012; 7: e41930.

[20] Zhao M, Zhang Y, Cai W, Li J, Zhou F, Cheng N, Ren R, Zhao C, Li X, Ren S, Zhou C and Hirsch FR. The Bim deletion polymorphism clinical profile and its relation with tyrosine kinase inhibitor resistance in Chinese patients with non-small cell lung cancer. Cancer 2014; 120: 2299-2307.

[21] Ng KP, Hillmer AM, Chuah CT, Juan WC, Ko TK, Teo AS, Ariyaratne PN, Takahashi N, Sawada K, Fei Y, Soh S, Lee WH, Huang JW, Allen JC Jr, Woo XY, Nagarajan N, Kumar V, Thalamuthu A, Poh WT, Ang AL, Mya HT, How GF, Yang LY, Koh LP, Chowbay B, Chang CT, Nadarajan VS, Chng WJ, Than H, Lim LC, Goh YT, Zhang S, Poh D, Tan P, Seet JE, Ang MK, Chau NM, Ng QS, Tan DS, Soda M, Isobe K, Nothen MM, Wong TY, Shahab A, Ruan X, Cacheux-Rataboul V, Sung WK, Tan EH, Yatabe Y, Mano H, Soo RA, Chin TM, Lim WT, Ruan Y and Ong ST. A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. Nat Med 2012; 18: 521-528.

[22] Winther Larsen A, Nissen PH, Meldgaard P, Weber B and Sorensen BS. EGFR CA repeat polymorphism predict clinical outcome in EGFR mutation positive NSCLC patients treated with erlotinib. Lung Cancer 2014; 85: 435-41.

[23] Corti A, Franzini M, Paolicchi A and Pompella A. Gamma-glutamyltransferase of cancer cells at the crossroads of tumor progression, drug resistance and drug targeting. Anticancer Res 2010; 30: 1169-1181.

[24] Oguztuzun S, Aydin M, Demirag F, Yazici U, Ozhavzali M, Kilic M and Iscan M. The expression of GST isoenzymes and p53 in non-small cell lung cancer. Folia Histochem Cytobiol 2010; 48: 122-127.

[25] Xu C, Feng D, Li L, Yu P, Hu X and Liu Y. [The expression and prognostic significance of ERCC1 and GST-pi in lung cancer]. Zhongguo Fei Ai Za Zhi 2010; 13: 195-200.

[26] Grimminger PP, Maus MK, Schneider PM, Metzger R, Holscher AH, Sugita H, Danenberg PV, Alakus H and Brabender J. Glutathione S-transferase PI (GST-PI) mRNA expression and DNA methylation is involved in the pathogenesis and prognosis of NSCLC. Lung Cancer 2012; 78: 87-91.

[27] Zhang R, Kang KA, Piao MJ, Kim KC, Zheng J, Yao CW, Cha JW, Maeng YH, Chang WY, Moon PG, Baek MC and Hyun JW. Epigenetic alterations are involved in the overexpression of glutathione S-transferase pi-1 in human colorectal cancers. Int J Oncol 2014; 45: 1275-1283.

[28] Sawers L, Ferguson MJ, Ihrig BR, Young HC, Chakravarty P, Wolf CR and Smith G. Glutathione S-transferase P1 (GSTP1) directly influences platinum drug chemosensitivity in ovarian tumour cell lines. Br J Cancer 2014; 111: 1150-8.

[29] Zhong SL, Zhou SF, Chen X, Chan SY, Chan E, Ng KY, Duan W and Huang M. Relationship between genotype and enzyme activity of glutathione S-transferases M1 and P1 in Chinese. Eur J Pharm Sci 2006; 28: 77-85.

[30] Jun L, Haiping Z and Beibei Y. Genetic polymorphisms of GSTP1 related to response to 5-FU-oxaliplatin-based chemotherapy and clinical outcome in advanced colorectal cancer patients. Swiss Med Wkly 2009; 139: 724-728.

[31] Sun N, Sun X, Chen B, Cheng H, Feng J, Cheng L and Lu Z. MRP2 and GSTP1 polymorphisms and chemotherapy response in advanced non-small cell lung cancer. Cancer Chemother Pharmacol 2010; 65: 437-446.

[32] Liu S, Yi Z, Ling M, Shi J, Qiu Y and Yang S. Predictive potential of ABCB1, ABCC3, and GSTP1 gene polymorphisms on osteosarcoma survival after chemotherapy. Tumour Biol 2014; 35: 9897-904.

[33] Rymaszewski AL, Tate E, Yimbesalu JP, Gelman AE, Jarzembowski JA, Zhang H, Pritchard KA Jr, Vikis HG. The role of neutrophil myeloperoxidase in models of lung tumor development. Cancers (Basel) 2014; 6: 1111-1127.

[34] Piedrafita FJ, Molander RB, Vansant G, Orlova EA, Pfahl M and Reynolds WF. An Alu element in the myeloperoxidase promoter contains a composite SP1-thyroid hormone-retinoic acid response element. J Biol Chem 1996; 271: 14412-14420.

[35] Li J, Fu Y, Zhao B, Xiao Y and Chen R. Myeloperoxidase G463A polymorphism and risk of lung cancer. Tumour Biol 2014; 35: 821-829.

[36] Gustafson HL, Yao S, Goldman BH, Lee K, Spier CM, LeBlanc ML, Rimsza LM, Cerhan JR, Habermann TM, Link BK, Maurer MJ, Slager SL, Persky DO, Miller TP, Fisher RI, Ambrosone

CB and Briehl MM. Genetic polymorphisms in oxidative stress-related genes are associated with outcomes following treatment for aggressive B-cell non-Hodgkin lymphoma. Am J Hematol 2014; 89: 639-645.

[37] Sedlak TW, Saleh M, Higginson DS, Paul BD, Juluri KR and Snyder SH. Bilirubin and glutathione have complementary antioxidant and cytoprotective roles. Proc Natl Acad Sci U S A 2009; 106: 5171-5176.

[38] Kapitulnik J and Maines MD. Pleiotropic functions of biliverdin reductase: cellular signaling and generation of cytoprotective and cytotoxic bilirubin. Trends Pharmacol Sci 2009; 30: 129-137.

[39] Kim SS, Seong S, Lim SH and Kim SY. Targeting biliverdin reductase overcomes multidrug resistance in leukemia HL60 cells. Anticancer Res 2013; 33: 4913-4919.

[40] Kim SS, Seong S, Lim SH and Kim SY. Biliverdin reductase plays a crucial role in hypoxia-induced chemoresistance in human glioblastoma. Biochem Biophys Res Commun 2013; 440: 658-663.

[41] Lin R, Wang X, Zhou W, Fu W, Wang Y, Huang W and Jin L. Association of a BLVRA common polymorphism with essential hypertension and blood pressure in Kazaks. Clin Exp Hypertens 2011; 33: 294-298.

Table S1. Candidate genes and SNPs included in the study

Gene	SNP	Chromosome	Rare allele	Common allele	MAF	Function	HWE-*P*
SOD2	rs4880	6	C	T	0.15	Val16Ala	0.284
GSTP1	rs1695	11	G	A	0.21	Ile105Val	0.787
TXN2	rs2281082	22	G	T	0.29	Intronic	0.104
PPARG*	rs1801282	3	G	C	0.07	Pro12Ala	< 0.001
GPX1	rs1800668	3	T	C	0.09	Promoter	0.339
CAT	rs769214	11	A	G	0.29	5' UTR	0.087
PON1	rs662	7	A	G	0.35	Q192R	0.491
NOS2	rs2297518	17	A	G	0.18	Ser608Leu	0.410
NOS3	rs1799983	7	T	G	0.14	Glu298Asp	0.829
MPO	rs2333227	17	G	A	0.16	Promoter	0.426
GPX1	rs1050450	3	T	C	0.11	Pro200Leu	0.614
NQO1	rs1800566	16	T	C	0.49	Pro187Ser	0.067
CYBA	rs4673	16	T	C	0.08	His72Tyr	0.209
AKR1C3*	rs1937840	10	C	G	0.19	Intronic	< 0.001
RAC1	rs10951982	7	A	G	0.20	Intronic	0.092
KL	rs3752472	13	T	C	0.10	Pro514Ser	0.497
BLVRA	rs699512	7	G	A	0.32	Thr3Ala	1.000
GPX4	rs713041	19	T	C	0.46	3' UTR	0.090
GCLC	rs17883901	6	T	C	0.12	Promoter	0.990
MT2A	rs10636	16	C	G	0.32	3' UTR	0.910

MAF: minor allele frequency; HWE: Hardy-Weinberg equilibrium. *indicates SNPs deviated from Hardy-Weinberg equilibrium and thus excluded from the analysis.

Table S2. Associations between SNPs and progression-free and overall survival of NSCLC patients

Gene	SNP	Progression-free survival		Overall survival	
		HR (95% CI)	*P* value	HR (95% CI)	*P* value
SOD2	rs4880	0.819 (0.601-1.114)	0.203	1.158 (0.859-1.560)	0.336
GSTP1	rs1695	1.293 (0.967-1.730)	0.083	0.679 (0.510-0.904)	0.008
TXN2	rs2281082	1.171 (0.933-1.471)	0.174	0.936 (0.752-1.165)	0.553
GPX1	rs1800668	1.224 (0.830-1.804)	0.308	0.848 (0.594-1.209)	0.361
CAT	rs769214	1.009 (0.797-1.277)	0.941	1.035 (0.820-1.308)	0.770
PON1	rs662	1.012 (0.803-1.274)	0.921	1.085 (0.861-1.367)	0.489
NOS2	rs2297518	0.934 (0.688-1.267)	0.659	0.776 (0.579-1.040)	0.090
NOS3	rs1799983	0.736 (0.537-1.009)	0.057	1.168 (0.842-1.620)	0.354
MPO	rs2243828	0.934 (0.702-1.242)	0.639	0.685 (0.519-0.904)	0.007
GPX1	rs1050450	1.146 (0.793-1.658)	0.468	1.193 (0.838-1.697)	0.327
NQO1	rs1800566	0.951 (0.759-1.190)	0.659	0.932 (0.801-1.085)	0.366
CYBA	rs4673	1.349 (0.895-2.035)	0.153	0.783 (0.556-1.101)	0.159
RAC1	rs10951982	1.173 (0.862-1.596)	0.310	0.972 (0.730-1.296)	0.848
KL	rs3752472	0.901 (0.608-1.335)	0.603	1.113 (0.772-1.606)	0.565
BLVRA	rs699512	0.969 (0.763-1.231)	0.799	0.786 (0.628-0.985)	0.036
GPX4	rs713041	0.874 (0.714-1.069)	0.190	0.903 (0.735-1.108)	0.329
MT2A	rs10636	1.042 (0.827-1.314)	0.726	1.000 (0.794-1.262)	0.997
GCLC	rs17883901	0.968 (0.684-1.372)	0.857	1.145 (0.868-1.511)	0.338