Association of EGFR mutations and HMGB1 genetic polymorphisms in lung adenocarcinoma patients

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Abstract

High-mobility group protein box 1 (HMGB1) is overexpressed and reported to be a prognostic factor in patients with non-small-cell lung cancer (NSCLC). Epidermal growth factor receptor (EGFR) mutants play an important role in NSCLC progression. The aim of this study was to explore potential associations between genetic polymorphisms of HMGB1 and EGFR mutations in a cohort that included 280 patients with NSCLC, some of whom were smokers and others who never smoked. Four tagged single-nucleotide polymorphisms (SNPs) of HMGB1 were detected by a TaqMan-based real-time polymerase chain reaction (PCR) in patients. We found that after adjusting for other covariates, NSCLC patients who smoked and who respectively had CG, CT, and TC heterozygotes of HMGB1 rs2249825, rs1045411, and rs1360485, were at lower risk of developing mutant EGFR, compared to those patients with wild-type homozygotes. Moreover, significant inverse associations between the CG and CG + GG genotypes of HMGB1 rs2249825 and the EGFR hotspot mutation, an exon 19 in-frame deletion, were also observed among NSCLC patients. Within patients harboring mutant EGFR, HMGB1 rs1360485 C (TC + CC) allele carriers were at higher risk of developing poorly differentiated cancer types (odds ratio=5.493, 95% confidence interval: 1.130–26.696, p=0.019), compared to patients with TT homozygotes. Furthermore, we found that HMGB1 rs1360485 polymorphisms seemed to be related to susceptibility to developing poorly differentiated cancer linked to tobacco consumption in EGFR mutant patients. In conclusion, our results suggested that HMGB1 variants are significantly inversely associated with EGFR mutations among NSCLC patients who smoked. HMGB1 variants and tobacco consumption might contribute to the pathological development of NSCLC.

Key words: High-mobility group protein box 1, Polymorphism, Susceptibility, Epidermal growth factor receptor, Non-small-cell lung cancer

Introduction

Lung cancer is one of the most prevalent cancers globally and is by far the leading cause of death among males and females worldwide [1]. Small-cell lung cancer (SCLC) and non-SCLC (NSCLC) are two
principal groups of lung cancer; the latter type comprises of 85%~90% of lung cancer diagnoses. NSCLC mainly includes the histologic types of adenocarcinoma (ADC) and squamous cell carcinoma (SCC) [2]. During the last decade, there have been major breakthroughs in many aspects of diagnosing and treating NSCLC, including surgery, chemotherapy, radiation therapy, and targeted drug therapy. However, understanding of the mechanisms underlying NSCLC development and progression remains limited, and effective indicators of an early diagnosis of NSCLC are still lacking. Among the many risk factors known to be associated with a higher lung cancer risk, tobacco smoking appears to have the strongest association [3]. Only about 10%~15% of lung cancer cases occur in people who have never smoked, which indicates that other factors such as genetic polymorphisms may play a role in determining disease risk and prognosis in lung cancer [4]. About 8% of patients have lung cancer solely due to inherited factors, and the contribution of hereditary factors in determining disease risk is also supported by statistics indicating a 2.4-fold increased lung cancer risk in people who are direct relatives of lung cancer patients [5].

The epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor encoded by a gene located on the short arm of chromosome 7. Autophosphorylation of EGFR tyrosine kinase domains leads to activation of downstream proteins that mediate cell proliferation and cell survival [6] and was reported to play an important role in tumorigenesis and progression of lung adenocarcinomas [7]. The EGFR gene was reported to harbor mutations and/or polymorphisms that increase susceptibility to lung cancer [8]. Most of these mutations/polymorphisms exist within the catalytic kinase domain, which increases EGFR activity. The most common somatic hotspot mutations in the tyrosine kinase domain of the EGFR gene are the in-frame deletion in exon 19 and a substitution mutation of lysine for arginine at amino acid position 858 (L858R) in exon 21. Mutations of L858R and the in-frame deletion in exon 19 were found to be more frequent in adenocarcinoma than other NSCLC and were suggested to promote cell viability [7]. Among adenocarcinoma patients, these EGFR mutations are more common in Asian patients, female patients, and patients who have never smoked [9]. Recently, genetic polymorphisms associated with susceptibility to various somatic mutations were described, including EGFR mutations [10-12], implying that EGFR mutations are linked to an individual’s genetic background. In addition, genomic DNA is continuously being damaged by mutagens from external agents, such as tobacco smoke in the environment [13]. In the human body, carcinogen-associated DNA damage must be repaired to maintain correct genetic information. If damaged DNA cannot be repaired, DNA lesions will form, such as EGFR mutations.

High-mobility group box 1 (HMGB1) is a highly conserved nuclear protein widely expressed in mammalian cells, and it plays a critical role in transcriptional regulation and chromatin construction. Loss of HMGB1 can increase DNA damage caused by potent carcinogens in cigarette smoke (such as benzo[a]pyrene diol epoxide) [14] and chemotherapeutic drugs (such as cisplatin) [15]. The binding of HMGB1 to DNA lesions can facilitate DNA repair, the response to DNA damage, and damage-induced chromatin remodeling; thus, it might prevent a carcinogenic or mutagenic outcome after exposure to DNA-damaging agents [16]. In addition to the nuclear function of HMGB1, HMGB1 was reported to be released into the extracellular matrix, where it exerts crucial functions in immunity, inflammation, and carcinogenesis through its diverse receptors such as the receptor for advanced glycation end-products (RAGE), the toll-like receptor (TLR) 2, and TLR 4 [17]. HMGB1 plays a pivotal role in the diagnosis and prognosis of NSCLC, especially with adenocarcinomas [18]. HMGB1 was upregulated in the serum of progressive NSCLC patients and associated with shorter overall survival and disease-free survival times. Overexpression of HMGB1 correlates with the proliferation, metastasis, and chemotherapy resistance of lung adenocarcinomas. HMGB1 may serve as an important risk factor for the development of lung cancer [19].

Investigation of polymorphisms of HMGB1 genes may contribute to our understanding of how cigarette smoke-derived carcinogen metabolism and DNA repair mechanisms affect the development of EGFR mutations in NSCLC. In this study, four polymorphisms of HMGB1 genes, including rs1412125, rs2249825, rs1045411, and rs1360485, were examined to study their associations with susceptibility to EGFR mutations in patients with lung adenocarcinoma.

**Material and Methods**

**Patient characteristics and consent**

Between 2012 and 2015, 280 lung adenocarcinoma patients with wild-type EGFR (67 men and 44 women; mean age = 65.36 ± 13.42 years) or a mutant EGFR (60 men and 109 women; mean age = 65.76 ± 13.57 years) were consecutively recruited from Taichung Cheng-Ching General Hospital.
(Taichung, Taiwan). Written ethical consent was obtained from all patients. The study was approved by the Institutional Review Board of Cheng-Ching General Hospital. Data obtained from medical records of each patient included demographics (age and sex), lifestyle variables (tobacco smoking status), and tumor stage and differentiation. Clinical information of patients was staged at the time of diagnosis following the tumor/node/metastasis staging system of the American Joint Committee on Cancer (AJCC).

Selection of single nucleotide polymorphisms (SNPs) of the HMGB1 gene

We genotyped four HMGB1 SNPs including rs1412125 (-1615T/C; promoter region), rs2249825 (3814C/G; intron 1), rs1360485 (T/C; 3’ untranslated region (3’ UTR)) and rs1045411 (2262C/T; 3’ UTR) selected according to Chinese HapMap data. Minor allelic frequencies of these SNPs were all >5%. These four polymorphisms are well defined and have been widely evaluated the association with a broad range of cancers such as oral squamous cell carcinoma, hepatocellular carcinoma, cervical cancer, colorectal cancer, breast cancer and lung cancer, especially in Han Chinese population [20]. In addition, the minor allele frequencies of these SNPs were all ≥5%.

Genomic DNA extraction from blood and HMGB1 genotyping

Genomic DNA was extracted from preserved whole blood in EDTA anti-coagulant tubes using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s protocol as previously described [21-23]. The TaqMan SNP Genotyping Assay with an ABI StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) was used to analyze allelic discrimination of HMGB1 SNPs. Allelic frequencies were determined using ABI SDS vers. 3.0 software.

Genomic DNA extraction from tumor tissues and EGFR gene sequencing

To acquire genomic DNA from tumor-frozen specimens and paraffin-embedded tissues, a QIAamp DNA Tissue kit (Qiagen) was used to extract genomic DNA according to the manufacturer’s protocol. DNA sequencing of the EGFR gene in exon 18-21 was amplified by a polymerase chain reaction (PCR), and finally subjected to a DNA sequencing reaction as described previously [9].

Statistical analysis

The distributions of genotype frequencies and demographic characteristics were compared between EGFR wild-type (WT) and mutation type in lung adenocarcinoma patients by using Mann–Whitney U test. Odds ratio (ORs) and their 95% confidence intervals (CIs) were assessed using logistic regression models. p<0.05 indicated statistically significant differences. Data were analyzed using SAS (version 9.1, 2005; SAS Institute Inc., Cary, NC).

Results

Demographic and clinical characteristics of patients with lung adenocarcinoma

Our study recruited 280 lung adenocarcinoma patients who were further divided into EGFR wild-type (WT) (n = 111; 39.6%) and mutation type (n = 169; 60.4%) groups. With regard to the EGFR mutation status, 79 subjects had L858R mutations, 81 had exon19 in-frame deletions, and nine had other mutations. The demographic, lifestyle, and clinical characteristics of both recruited groups with the WT and mutant EGFR are shown in Table 1. Between these two groups, there was no significant difference in the distribution of age, clinical stage, or TNM stage. Compared to the group with the WT EGFR, subjects with the mutant EGFR were significantly observed in females (64.5% vs. 39.6%) and those who had never smoked (77.5% vs. 45.0%), and those who had more well-differentiated (12.4% vs. 7.2%) and moderately differentiated tumors (81.7% vs. 72.1%).

| Table 1. Demographics and clinical characteristics of 280 patients with lung adenocarcinoma and their epidermal growth factor mutation status |
| Variable | Wild type (N=111) n (%) | EGFR mutation (N=169) n (%) | p value |
| Age | 65.36 ± 13.42 | 65.76 ± 13.57 | 0.810 |
| Gender | | | |
| Male | 67 (60.4%) | 60 (35.5%) | <0.001 |
| Female | 44 (39.6%) | 109 (64.5%) | |
| Cigarette smoking status | | | |
| Never-smoker | 50 (45.0%) | 131 (77.5%) | 0.001 |
| Ever-smoker | 61 (55.0%) | 38 (22.5%) | |
| Stage | | | |
| I+II | 26 (23.4%) | 47 (27.8%) | 0.413 |
| III+IV | 85 (76.6%) | 122 (72.2%) | |
| Tumor T status | | | |
| T1+T2 | 60 (54.1%) | 108 (63.9%) | 0.100 |
| T3+T4 | 51 (45.9%) | 61 (36.1%) | |
| Lymph node status | | | |
| Negative | 29 (26.1%) | 54 (32.0%) | 0.296 |
| Positive | 82 (73.9%) | 115 (68.0%) | |
| Distant metastasis | | | |
| Negative | 54 (48.6%) | 80 (47.3%) | 0.830 |
| Positive | 57 (51.4%) | 89 (52.7%) | |
| Cell differentiation | | | |
| Well | 8 (7.2%) | 21 (12.4%) | <0.001 |
| Moderately | 80 (72.1%) | 138 (81.7%) | |
| Poorly | 23 (20.7%) | 10 (5.9%) | |

EGFR, epidermal growth factor receptor.
Associations between **HMGB1** genotypes and **EGFR** mutations in adenocarcinoma patients who did or did not smoke

Regardless of sex, cigarette smoking is a major risk factor for lung cancer, and cigarette smoking was reported to affect the EGFR status in lung adenocarcinoma patients [24]. In this study, we divided our recruited lung adenocarcinoma patients into smoking and non-smoking groups and further investigated the difference between HMGB1 polymorphisms and EGFR status in these two groups. Distributions of HMGB1 genetic polymorphisms in the promoter region (rs141215, −1615T/C), intron 1 (rs2249825, 3814C/G), and the 3’ UTR (rs1045411, 2262C/T and rs1360485, T/C) in patients with the WT or mutant EGFR are shown in Table 2. Alleles with the highest distribution frequency of rs141215, rs2249825, rs1045411, and rs1360485 in our recruited adenocarcinoma patients with the WT or mutant EGFR were homozygous for TT, CC, CC, and TT, respectively. For rs141215 polymorphisms, there was no significant association demonstrated between variant types (TC, CC, and TC+CC) and the EGFR mutation type. In the smoking group, subjects with the HMGB1 polymorphic rs2249825 C, rs1045411 CT, and rs1360485 T/C genotypes exhibited significantly (p<0.05) lower frequencies of 0.07- (95% CI: 0.01-0.74), 0.12- (95% CI: 0.02-0.69), and 0.12-fold (95% CI: 0.02-0.72) respectively, of having an EGFR mutation compared to their corresponding WT homozygotes (Table 2). In the non-smoking and overall cohorts we recruited, we observed that there was no significant difference in having an EGFR mutation in individuals with rs2249825 and rs1045411 polymorphisms of the HMGB1 gene compared to WT individuals (Table 2).

**Associations between HMGB1 SNPs and EGFR hotspot mutations among lung adenocarcinoma patients**

Two most common EGFR hotspot mutations, L858R and an in-frame deletion, were also observed in most (95%) of our recruited patients with EGFR mutations. We further assessed associations of the L858R mutation or in-frame deletion of the EGFR gene with HMGB1 genotypes. The CG and CG+GG genotypes of EGFR were significantly associated with a decreased proportion of in-frame deletions (adjusted odds ratio (AOR)=0.473, 95% CI: 0.228-0.979, p=0.044; AOR=0.463, 95% CI: 0.227-0.941, p=0.033), but not with the L858R mutation (Table 3). In contrast, no significant association was observed of HMGB1 in patients with lung adenocarcinoma and multiple logistic regression analysis of epidermal growth factor receptor mutations associated with the cigarette smoking status.

**Correlations between polymorphic genotypes of HMGB1 and the clinical status of lung adenocarcinoma patients with EGFR mutations**

Impacts of the variant HMGB1 genotypes on the clinicopathological development of NSCLC harboring EGFR mutations, such as clinical cancer stage, primary tumor size, lymph node, distant metastasis,

**Table 2. Distribution frequency of high-mobility group box 1 (HMGB1) genotypes of patients with lung adenocarcinoma and multiple logistic regression analysis of epidermal growth factor receptor mutations associated with the cigarette smoking status.**

| SNP genotype | All cases (N=280) | Non-smokers (N=181) | Smokers (N=99) |
|--------------|------------------|---------------------|----------------|
|              | Wild type (N=111) | AOR (95% CI) | Wild type (N=131) | AOR (95% CI) | Wild type (N=61) | AOR (95% CI) |
| rs141215     |                   |                     |                 |                     |                     |             |
| TT           | 59 (53.2%)        | 89 (52.7%)         | 1.00            | 28 (56.0%)          | 67 (51.1%)         | 1.00          |
| TC           | 43 (38.7%)        | 66 (39.1%)         | 0.82 (0.44-1.54)| 20 (40.0%)          | 53 (40.5%)         | 0.76 (0.29-1.95) |
| CC           | 9 (8.1%)          | 14 (8.2%)          | 0.95 (0.27-3.32)| 2 (4.0%)           | 11 (8.4%)          | 3.55 (0.28-45.12) |
| TC+CC        | 52 (46.8%)        | 80 (47.3%)         | 0.84 (0.46-1.54)| 22 (44.0%)          | 64 (48.9%)         | 0.89 (0.35-2.22) |
| rs2249825    |                   |                     |                 |                     |                     |             |
| CC           | 74 (66.7%)        | 127 (75.1%)        | 1.00            | 39 (78.0%)          | 95 (72.5%)         | 1.00          |
| CT           | 34 (30.6%)        | 57 (33.0%)         | 0.55 (0.27-1.10)| 10 (20.0%)          | 33 (25.2%)         | 1.19 (0.41-3.50) |
| TT           | 5 (4.4%)          | 11 (6.5%)          | 1.02 (0.33-3.81)| 2 (4.0%)           | 3 (2.3%)           | 1.52 (0.09-27.00) |
| rs1045411    |                   |                     |                 |                     |                     |             |
| CC           | 42 (33.3%)        | 71 (42.9%)         | 0.63 (0.32-1.23)| 11 (22.0%)          | 36 (27.5%)         | 1.22 (0.43-3.45) |
| TC           | 41 (36.9%)        | 65 (39.3%)         | 0.60 (0.30-1.15)| 12 (23.8%)          | 42 (31.5%)         | 0.51 (0.28-0.90) |
| TT           | 17 (13.8%)        | 33 (19.8%)         | 1.72 (0.77-3.87)| 4 (8.0%)           | 6 (4.5%)           | 1.44 (0.32-6.78) |
| rs1360485    |                   |                     |                 |                     |                     |             |
| CC           | 62 (55.9%)        | 103 (60.9%)        | 1.00            | 33 (66.0%)          | 76 (58.0%)         | 1.00          |
| CT           | 41 (36.9%)        | 65 (39.3%)         | 0.60 (0.30-1.15)| 12 (23.8%)          | 42 (31.5%)         | 0.51 (0.28-0.90) |
| TT           | 8 (7.2%)          | 15 (8.6%)          | 1.02 (0.33-3.81)| 2 (4.0%)           | 3 (2.3%)           | 1.52 (0.09-27.00) |
| CG+GG        | 37 (33.3%)        | 54 (32.4%)         | 0.63 (0.32-1.23)| 11 (22.0%)          | 36 (27.5%)         | 1.22 (0.43-3.45) |

The adjusted odds ratios (AORs) with 95% confidence intervals (CIs) were estimated by multiple logistic regression models after controlling for age and gender.

Note: Bold text indicates a significant association with p value of <0.05.

Abbreviations: SNP, single-nucleotide polymorphism.
and degree of tumor cell differentiation, were further evaluated, and results are shown in Table 4. We classified patients into two subgroups of patients who had the homozygous WT alleles or who had at least one polymorphic allele. Among 169 lung adenocarcinoma patients with an EGFR mutation who carried at least one polymorphic C allele of HMGB1 rs1360485, they showed a significantly higher risk of having a poorly differentiated carcinoma (5.493-fold, 95% CI: 1.130–26.696, p=0.019) than those carrying the WT gene (Table 4). Next, we analyzed the synergistic effects of cigarette smoking combined with rs1360485 SNPs in lung adenocarcinoma patients with an EGFR mutation. Sixty patients who smoked cigarettes and harbored at least one polymorphic C allele of HMGB1 rs1360485 had an enhanced risk (9.9-fold, 95% CI: 1.109–88.339, p=0.016) for developing poorly differentiated tumors compared to patients with TT homozygotes (Table 4). However, no significant associations between rs1360485 gene polymorphisms and the clinicopathologic status were observed in 109 non-smoking patients with EGFR mutations.

### Table 3. Associations between the polymorphisms of high-mobility group box 1 (HMGB1) and the epidermal growth factor receptor hotspot mutations in lung adenocarcinoma patients.

| Variable | Wild type | L858R | Exon 19 in-frame deletion |
|----------|-----------|-------|-------------------------|
|          | (N=111) (%) | (N=79) (%) | AOR (95% CI) | (N=81) (%) | AOR (95% CI) |
| rs1412125 |           |       |                        |           |               |
| TT       | 59 (53.2%) | 39 (49.4%) | 1.00 | 46 (56.8%) | 1.00 |
| TC       | 43 (38.7%) | 34 (43.0%) | 1.03 (0.522–2.044) | 28 (34.6%) | 0.640 (0.328–1.250) |
| CC       | 9 (8.1%)   | 6 (7.6%) | 1.109 (0.319–3.852) | 7 (8.6%) | 0.870 (0.290–2.608) |
| CT+CC    | 52 (46.8%) | 40 (50.6%) | 1.045 (0.545–2.003) | 35 (43.2%) | 0.683 (0.366–1.272) |
| rs2249825 |           |       |                        |           |               |
| CC       | 74 (66.7%) | 55 (69.6%) | 1.00 | 65 (80.2%) | 1.00 |
| CG       | 34 (30.6%) | 21 (26.6%) | 0.767 (0.370–1.591) | 15 (18.5%) | 0.473 (0.228–0.979)* |
| GG       | 3 (2.7%)   | 3 (3.8%) | 1.234 (0.196–7.757) | 1 (1.2%) | 0.349 (0.032–3.811) |
| CG+GG    | 37 (33.3%) | 24 (30.4%) | 0.804 (0.397–1.626) | 16 (19.8%) | 0.463 (0.227–0.941)* |
| rs1045411 |           |       |                        |           |               |
| TT       | 62 (55.9%) | 44 (55.7%) | 1.00 | 52 (64.2%) | 1.00 |
| CT       | 41 (36.9%) | 29 (36.7%) | 0.913 (0.457–1.823) | 26 (32.1%) | 0.766 (0.402–1.459) |
| TT       | 8 (7.2%)   | 6 (7.6%) | 0.963 (0.275–3.370) | 3 (3.7%) | 0.448 (0.106–1.884) |
| CT+TT    | 49 (44.1%) | 35 (44.3%) | 0.921 (0.477–1.779) | 29 (35.8%) | 0.715 (0.385–1.330) |
| rs1360485 |           |       |                        |           |               |
| TT       | 60 (54.1%) | 42 (53.2%) | 1.00 | 46 (56.8%) | 1.00 |
| CC       | 40 (36.0%) | 31 (39.2%) | 0.989 (0.493–1.986) | 31 (38.3%) | 1.042 (0.549–1.977) |
| CT       | 11 (9.9%)  | 6 (7.6%) | 0.843 (0.252–2.620) | 4 (4.9%) | 0.498 (0.140–1.769) |
| CT+CC    | 51 (45.9%) | 37 (46.8%) | 0.961 (0.496–1.864) | 35 (43.2%) | 0.929 (0.504–1.714) |

The adjusted odds ratios (AORs) with 95% confidence intervals (CIs) were estimated by multiple logistic regression models after controlling for age and gender. Note: Bold text indicates a significant association with a p value of <0.05. Abbreviations: SNP, single-nucleotide polymorphism.

### Table 4. Associations between polymorphic genotypes of high-mobility group box 1 (HMGB1) (rs1360485) and clinicopathologic characteristics of lung cancer with epidermal growth factor receptor (EGFR) mutations.

| Variable                  | EGFR mutation (N=169) | EGFR mutation with non-smoking (N=109) | EGFR mutation with smoking (N=60) |
|---------------------------|------------------------|----------------------------------------|----------------------------------|
|                           | AOR (95% CI)           | AOR (95% CI)                           | AOR (95% CI)                     |
|                           | TT (N=94)              | TT+CC (N=75)                           | TT (N=34)                       |
| Stage                     |                        |                                        |                                 |
| I+II                      | 30 (51.9%)             | 20 (33.3%)                             | 5 (19.2%)                       | 0.367 |
| III+IV                    | 64 (68.1%)             | 40 (66.7%)                             | 24 (70.6%)                      | 0.808 |
| Tumor T status            |                        |                                        |                                 |
| T1+T2                     | 62 (66.0%)             | 40 (66.7%)                             | 22 (64.7%)                      | 0.252 |
| T3+T4                     | 32 (34.0%)             | 20 (33.3%)                             | 12 (35.3%)                      | 0.500 |
| Lymph node status         |                        |                                        |                                 |
| Negative                  | 34 (36.2%)             | 24 (40.0%)                             | 10 (29.4%)                      | 0.582 |
| Positive                  | 60 (63.8%)             | 36 (60.0%)                             | 24 (70.6%)                      | 0.500 |
| Distant metastasis        |                        |                                        |                                 |
| Negative                  | 47 (50.0%)             | 32 (53.3%)                             | 15 (44.1%)                      | 0.889 |
| Positive                  | 47 (50.0%)             | 28 (46.7%)                             | 19 (55.9%)                      | 0.577 |
| Cell differentiation      |                        |                                        |                                 |
| Well + Moderately         | 92 (97.9%)             | 59 (98.3%)                             | 33 (97.1%)                      | 0.016* |
| Poorly                    | 2 (2.1%)               | 1 (1.7%)                               | 1 (2.9%)                       | 0.231 |

*p value of <0.05 as statistically significant.

Odd ratio (OR) (95% confidence interval (CI)): 5.493 (1.130–26.696); OR (95% CI): 9.900 (1.109–88.339).
Discussion

Increasing evidence has shown that HMGB1 plays a key role in various cancer types including NSCLC [18], and HMGB1 dysfunction affects tumor development and therapy through regulating several hallmarks of cancer, such as metastasis, angiogenesis, resisting cell death, genome instability, mutations, and so on [15]. HMGB1 was characterized as having both oncogenic and tumor-suppressive roles in promoting both cell survival and death by regulating multiple signaling pathways, including genome stability, inflammation, immunity, proliferation, apoptosis, autophagy, metastasis, and metabolism [15, 17]. Moreover, the HMGB1 expression level was reported to be inversely correlated with tumor differentiation [25]. In the past few years, several studies evaluated the association of HMGB1 polymorphisms with cancer susceptibility. In total, four frequently used SNPs within the human HMGB1 gene, rs1412125, rs2249825, rs1045411, and rs1360485, were studied for their associations with various cancer types including oral [26], liver [27], cervical [28], colorectal [29], and lung [30] cancers, especially in Chinese Han population [20]. These four HMGB1 SNPs with minor allele frequencies were of 0.05 (5%) or greater in the Chinese Han Beijing population. Moreover, previous GWAS study indicated that an association might exist between rs1360485 and rs1412125 SNPs with lung cancer risk [31] and rs1360485 showed a strong linkage disequilibrium with rs1045411 and rs2249825 [30, 32]. In lung cancer, knockdown of HMGB1 can increase the chemosensitivity of cells [33]. Moreover, the rs1412125 and rs2249825 polymorphisms were recently reported to be associated with the response to platinum-based chemotherapy in Chinese population [34]. High frequency of EGFR mutations in NSCLC was found in Asian population and the EGFR mutation of NSCLC was demonstrated to be a predictor of responsiveness to targeted therapy. Overexpression of HMGB1 was reported to promote cell proliferation and invasion in EGFR-mutant lung adenocarcinoma cells [35]. Based on the above annotations, we first investigated the association of EGFR mutation statuses in lung adenocarcinoma with variations (rs1412125, rs2249825, rs1045411, and rs1360485) of HMGB1 and their interactions with subjects who smoke in a Taiwanese population. In this study, our recruited cohort revealed a high prevalence of EGFR mutations in females and subjects who had never smoked and who harbored a more-differentiated tumor type. Characteristics of our recruited cohort were similar to Asian NSCLC patients as previously reported [36]. As to correlations between HMGB1 SNPs and frequencies of EGFR mutations, variant types (CG or CG + GG) of HMGB1 3814C/G polymorphism (rs2249825) were significantly negatively correlated with the EGFR mutation type of an exon 19 in-frame deletion. Furthermore, we observed that the rs2249825 C/G, rs1045411 C/T, and rs1045411 T/C heterozygous polymorphisms were associated with a significantly lower proportion of developing EGFR mutations compared to their respective homozygous C/C, C/C, and T/T polymorphisms in the smoking population. These results suggested that the respective G, T, and C alleles of rs2249825, rs1045411, and rs1360485 might be protective factors against developing EGFR mutations in individuals who smoke. Moreover, individuals who smoke and carry at least one C allele of rs1360485 had a higher risk of having a poorly differentiated tumor type. Cigarette smoking, the best-known causative factor of lung cancer, was reported to induce HMGB1 upregulation in the blood and lung tissues of smokers [37]. A carcinogen of cigarette smoke, benzo[a]pyrene, can cause DNA damage, and base excision repair is a critical repair pathway that removes benzo[a]pyrene-induced DNA damages [38]. It was postulated that impaired base excision repair may be associated with increased genomic instability and increased tumor mutation rates such as EGFR mutations, and HMGB1 was reported to play an important role in base excision repair [39]. Based on these findings, we suggest that HMGB1 SNPs might affect cigarette smoke-induced HMGB1 expression to further decrease genomic instability and tumor EGFR mutations. Actually, expression of another base excision repair gene, excision repair cross complementing 1, was reported to be inversely correlated with EGFR mutations in NSCLC patients [40].

Previous studies demonstrated that polymorphisms of certain genes can influence gene expressions and biological functions. The C-to-G variation of the rs2249825 polymorphism, located in intron 1, might affect the binding site of v-myb and provide transcriptional control to enhance HMGB1 expression [34]. The rs2249825 polymorphism was also correlated with lipopolysaccharide-induced HMGB1 production in neutrophils [41]. In addition to rs2249825, two closely related SNPs, rs1045411 and rs1360485, showed strong linkage, are located in the 3’ UTR of the HMGB1 gene, and may alter HMGB1 messenger (m)RNA expression through micro (mi)RNA binding. For example, rs1045411 is in close proximity to the mir-505 binding site, and a significant influence of CT/CC genotypes of rs1045411 was reported to alter HMGB1 gene.
expression by regulating miR-505-binding activity [32]. Until now, the rs2249825 C/G polymorphism was reported to enhance HMGB1 expression, and the functional role of this SNP might have prevented EGF mutations in our study cohort by maintaining genomic stability. In smokers with EGF-mutant adenocarcinoma, not only rs2249825, but also rs1045411 and rs1360485 SNPs were all correlated with lower incidences of EGF mutations. Although the rs1045411 SNP was reported to decrease HMGB1 expression by miR-505 [32], cigarette smoking might influence other miRNAs [42, 43] to regulate HMGB1 expression. Moreover, rs1360485 polymorphisms, TC and CC genotypes, were reported to reduce risk of lung adenocarcinoma, but there was no additive interaction between rs1360485 polymorphisms and tobacco smoking [31]. Until now, interactions among tobacco consumption, rs1045411 and rs1360485 polymorphisms, and HMGB1 expression are unclear and worthy of future investigations.

In conclusion, in a Taiwanese population, we first identified associations among HMGB1 genetic polymorphisms, cigarette smoking, and the EGF mutation status. Our results indicated that the exon 19 in-frame deletion mutation of the EGF was associated with polymorphisms of the HMGB1 gene related to carcinogen detoxification and DNA repair in lung adenocarcinoma patients who smoked. These findings provide novel insights into the genesis of EGF mutations and can be utilized as a predictor of responsiveness to EGF-targeted therapy.

Competing Interests

The authors have declared that no competing interest exists.

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