Genetic Associations of *Visfatin* Polymorphisms with EGFR Status and Clinicopathologic Characteristics in Lung Adenocarcinoma

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Abstract: Lung adenocarcinoma (LUAD) is the most common histologic type of lung cancer. Mutations of the *epidermal growth factor receptor* (EGFR) gene are among the most common genetic alterations in LUAD and are the targets of EGFR tyrosine kinase inhibitors. The enzyme *visfatin* is involved in the generation of the oxidized form of nicotinamide adenine dinucleotide (NAD⁺) and regulation of intracellular adenosine triphosphate (ATP), critical processes in cancer cell survival and growth. This study explored the relationship between *visfatin* single nucleotide polymorphisms (SNPs) with EGFR status and the clinicopathologic development of LUAD in a cohort of 277 Taiwanese men and women with LUAD. Allelic discrimination of four *visfatin* SNPs rs11977021, rs61330082, rs2110385 and rs4730153 was determined using a TaqMan Allelic Discrimination assay. We observed higher prevalence rates of advanced (T3/T4) tumors and distant metastases in EGFR wild-type patients carrying the rs11977021 CT + TT and rs61330082 GA + AA genotypes, respectively, compared with patients carrying the CC and GG genotypes. EGFR wild-type patients carrying the rs11977021 CT + TT genotypes were also more likely to develop severe (stage III/IV) malignancy compared with patients carrying the CC genotype. An analysis that included all patients found that the association persisted between the rs11977021 CT + TT and rs61330082 GA + AA genotypes and the development of T3/T4 tumors compared with patients carrying the rs11977021 CC and rs61330082 GG genotypes. In conclusion, these data indicate that *visfatin* SNPs may help to predict tumor staging in LUAD, especially in patients with EGFR wild-type status.

Keywords: *visfatin*; nicotinamide phosphoribosyltransferase; pre-B-cell colony-enhancing factor; single nucleotide polymorphism; non-small cell lung cancer

1. Introduction

Lung cancer has the highest mortality rate of all cancers, according to global and national statistics in Taiwan [1,2] (Ministry of Health and Welfare, Taiwan 2021). Based on histology, lung cancer can be divided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Lung adenocarcinoma (LUAD) is the most common subtype of...
NSCLC and of lung cancers overall [3]. Major risk factors for lung cancer include cigarette smoking, secondhand smoking, use of domestic biomass fuels, air pollution, pulmonary conditions such as chronic obstructive pulmonary disease, and genetic factors [4].

Epidermal growth factor receptor (EGFR) mutations in the tyrosine kinase domain are common in NSCLC patients, with higher rates particularly in those with adenocarcinoma, non-smokers, females, and Asian populations [5,6]. Around two-thirds (67.1%) of patients with EGFR-mutant NSCLC have classical EGFR mutations (exon 21 L858R point mutation or exon 19 deletions (Ex19del)) [7]. EGFR mutations in the tyrosine kinase domain increase EGFR kinase activity and signs of tumorigenesis such as cancer cell proliferation, migration, invasion, and angiogenesis [8,9]. Although no molecular-targeted therapy has shown any overall survival benefit in early-stage NSCLC with EGFR mutations [10], advanced-stage NSCLC harboring sensitizing EGFR-positive mutations responds well to tyrosine kinase inhibitors (TKIs) and these agents have proven more effective than the historical standard of care, platinum-based chemotherapy [8]. In patients with untreated, EGFR-mutated advanced NSCLC, overall survival is improved with the third-generation EGFR TKI osimertinib compared with the first-generation TKIs gefitinib and erlotinib [11].

Given the important role of EGFR mutation status in NSCLC development, we decided to explore interactions amongst crucial genes and EGFR status in NSCLC and LUAD. Interleukin-17A, the tumor suppressor protein WW domain-containing oxidoreductase, tissue inhibitor of metalloproteinase 3 (TIMP3), and long noncoding RNA H19 polymorphisms have all been associated with clinicopathologic characteristics in lung cancers [5,9,12,13]. These four studies have helped to identify subgroups of patients at high risk for LUAD progression.

Visfatin, a visceral fat-derived adipocytokine, exhibits identical properties to both the pre-B-cell colony enhancing factor (PBEF) molecule that is secreted by human peripheral blood lymphocytes [14] and the enzyme nicotinamide phosphoribosyltransferase (NAMPT), and is therefore capable of synthesizing nicotinamide adenine dinucleotide (NAD⁺), a key molecule involved in the generation of adenosine triphosphate (ATP) [15]. EGFR-mutated NSCLC depends on a large quantity of intracellular ATP for tumor progression, so visfatin is critical to the survival of EGFR-mutated NSCLC [16].

The aberrant secretion of visfatin is critical for obesity-associated cancers [17,18] and has been detected in tumor and plasma samples of pancreatic ductal adenocarcinoma, oral squamous cell carcinoma (OSCC), breast cancer, renal cell carcinoma, thyroid cancer and also NSCLC [17,19]. Plasma visfatin levels have been correlated with tumor, node, and metastasis (TNM) staging in gastric cancer and NSCLC, and with the depth of gastric cancer invasion [17]. High plasma visfatin levels are also a poor prognostic factor in hepatocellular carcinoma, breast cancer, gastric cancer, and urothelial carcinoma [18]. This evidence suggests that visfatin could be a potentially useful marker in clinical practice for cancer diagnosis, prognosis and even for cancer therapy [15,20,21]. In SCLC, high serum visfatin levels are associated with brain metastases and visfatin appears to promote SCLC cell migration across the blood–brain barrier [22]. A recently developed dual inhibitor of visfatin and EGFR has shown excellent antiproliferative activities in various cancer cell lines, including H1975 NSCLC cells harboring the EGFR\textsuperscript{L858R/T790M} mutation [21].

A previous study from our laboratory described how certain visfatin polymorphisms in a cohort of Taiwanese males were associated with higher or lower risks of developing OSCC [23]. In this study, we explored whether certain visfatin polymorphisms play a similar role in lung adenocarcinoma and act as potential diagnostic or therapeutic targets. Analyses specifically examined associations between four visfatin single nucleotide polymorphisms (SNPs) rs11977021, rs61330082, rs2110385, and rs4730153, which have been studied for their association with risk of developing various cancers [19,23–25], EGFR status and clinicopathologic characteristics in LUAD. Our results indicate that visfatin polymorphisms are associated with clinicopathologic staging in LUAD.
2. Materials and Methods

2.1. Patients

A total of 277 patients with LUAD harboring different \textit{EGFR} statuses and 277 case-controls with similar baseline characteristics (Table S1) were recruited from Chung Shan Medical University Hospital, Taichung, Taiwan. Medical records from each patient were reviewed and their clinicodemographic details were recorded (age, sex, smoking behavior, tumor staging, TNM classification and cell differentiation status). Clinical disease staging was determined according to the rules in the American Joint Committee on Cancer Staging Manual. Informed written consent was obtained from each patient prior to starting the study, and the study protocol was approved by the Institute Review Board of Chung Shen Medical University Hospital (No. CS1-20144).

2.2. Genomic DNA Extraction and \textit{EGFR} Gene Sequencing

Tumor DNA was extracted from paraffin-embedded tissues using the QIAmp DNA Mini Kit (Qiagen, Valencia, CA, USA), following the manufacturer’s protocols [26–28]. To classify the DNA samples as \textit{EGFR} wild-type or \textit{EGFR}-mutant status, L858R or exon 19 deletions (Ex19del), matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) was used, as previously described [9].

2.3. Genotyping of Visfatin Polymorphisms

Peripheral blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes and DNA was extracted using a QIAamp DNA blood mini kit (Qiagen, Valencia, CA, USA). Three of the four analyzed \textit{visfatin} SNPs, rs11977021, rs61330082 and rs2110385, are located in the promoter region. \textit{visfatin} rs4730153 is located at the intron region between exon six and seven. Allelic discrimination of four \textit{visfatin} SNPs: C and T (C/T) alleles of rs11977021, G/A alleles of rs61330082, G/T alleles of rs2110385, and G/A alleles of rs4730153 was performed using the TaqMan SNP Genotyping Assay and the ABI StepOne-Plus Real-Time PCR system (Applied Biosystems, Foster City, CA, USA), as previously described [23,28]. The context sequences of the four \textit{visfatin} SNP probes on the plus (sense) strand are shown in Table S2. The results of the replication plots performed by TaqMan genotyping assay in this study are shown in Figures S1–S4.

2.4. Statistical Analysis

All data were analyzed for statistical significance using SAS software, version 9.1 (SAS Institute Inc., Cary, NC, USA). Differences in demographic and clinical characteristics between \textit{EGFR} wild-type and \textit{EGFR}-mutant patients were calculated using the Mann–Whitney \textit{U} test and the Fisher’s exact test. Adjusted odds ratios and 95% confidence intervals (CIs) were estimated by multiple logistic regression models after controlling for age, sex, and cigarette smoking status. A \textit{p}-value of <0.05 was regarded as statistically significant.

3. Results

3.1. Baseline Characteristics of the Study Participants

This study recruited 277 Taiwanese men and women with LUAD who were categorized as either \textit{EGFR} wild-type (\(n=111\)) or \textit{EGFR} mutation-positive (\(n=166\)) and evaluated by age, sex, smoking history, LUAD stage and grade (Table 1). The mean age was 65.36 ± 13.42 years in the \textit{EGFR} wild-type group and 65.90 ± 13.64 years in the \textit{EGFR} mutation group, with no statistically significant between-group difference (Table 1). Significantly higher proportions of \textit{EGFR}-mutant patients were female versus male (64.5% vs. 35.5%, \(p < 0.001\)) and never-smokers versus ever-smokers (77.1% vs. 22.9%, \(p < 0.001\)) (Table 1). Tumor stage and TNM status did not differ significantly between the \textit{EGFR} wild-type and \textit{EGFR}-mutant groups. However, a comparison of tumor grade revealed that compared with the \textit{EGFR}-mutant patients, the \textit{EGFR} wild-type patients had a higher prevalence of poor cell differentiation (20.7% vs. 6.0%, \(p = 0.001\)) and lower rates of well-differentiated
cells (7.2% vs. 11.4%, respectively, \( p = 0.001 \)) and moderately differentiated cells (72.1% vs. 82.5%, \( p = 0.001 \)) (Table 1).

Table 1. Demographics, clinical characteristics and EGFR status of 277 lung adenocarcinoma patients.

| Variable                        | EGFR Wild-Type \((n = 111)\) n (%) | EGFR Mutation \((n = 166)\) n (%) | \( p \) Value |
|---------------------------------|------------------------------------|-----------------------------------|---------------|
| Age\(\text{ Mean} \pm \text{ SD}\) | 65.36 ± 13.42                      | 65.90 ± 13.64                     | \( p = 0.420 \) |
| Sex, \( n \) (%)                |                                    |                                   |               |
| Male                            | 67 (60.4%)                         | 59 (35.5%)                        | \( p < 0.001 \) |
| Female                          | 44 (39.6%)                         | 107 (64.5%)                       |               |
| Cigarette smoking status, \( n \) (%) |                                    |                                   |               |
| Never-smoker                    | 50 (45.0%)                         | 128 (77.1%)                       | \( p < 0.001 \) |
| Ever-smoker                     | 61 (55.0%)                         | 38 (22.9%)                        |               |
| Stage, \( n \) (%)              |                                    |                                   | \( p = 0.425 \) |
| I/II                            | 26 (23.4%)                         | 46 (27.7%)                        |               |
| III/IV                          | 85 (76.6%)                         | 120 (72.3%)                       |               |
| Tumor status, \( n \) (%)       |                                    |                                   | \( p = 0.083 \) |
| T1/T2                           | 60 (54.1%)                         | 107 (64.5%)                       |               |
| T3/T4                           | 51 (45.9%)                         | 59 (35.5%)                        |               |
| Lymph node status, \( n \) (%)  |                                    |                                   | \( p = 0.254 \) |
| Negative                        | 29 (26.1%)                         | 54 (32.5%)                        |               |
| Positive                        | 82 (73.9%)                         | 112 (67.5%)                       |               |
| Distant metastases, \( n \) (%) |                                    |                                   | \( p = 0.786 \) |
| Negative                        | 54 (48.6%)                         | 78 (47.0%)                        |               |
| Positive                        | 57 (51.4%)                         | 88 (53.0%)                        |               |
| Cell differentiation, \( n \) (%) |                                    |                                   | \( p = 0.001 \) |
| Well                            | 8 (7.2%)                           | 19 (11.4%)                        |               |
| Moderate                        | 80 (72.1%)                         | 137 (82.5%)                       |               |
| Poor                            | 23 (20.7%)                         | 10 (6.0%)                         |               |

Abbreviation: EGFR, epidermal growth factor receptor.

3.2. No Association of Visfatin SNP (rs11977021, rs61330082, rs2110385, and rs4730153) Distribution Frequency with EGFR Status or LUAD

To examine the potential association of the visfatin SNPs with LUAD, the genotype frequency of the four visfatin SNPs in 277 LUAD patients were compared with 277 case-controls. After adjusting for age, sex, and cigarette smoking status using multiple logistic regression models, none of the genotypes for the four visfatin SNPs were associated with LUAD (Table 2).

Table 2. Adjusted odds ratios (ORs) with their 95% confidence intervals (CIs) for lung adenocarcinoma associated with Visfatin genotype frequencies.

| Genotypes | Control \((n = 277)\) | LUAD \((n = 277)\) | AOR (95% CI) | \( p \) Value |
|-----------|-----------------------|---------------------|--------------|---------------|
| rs11977021 |                       |                     |              |               |
| CC        | 71 (25.6%)            | 72 (26.0%)          | 1.000 (reference) |               |
| CT        | 130 (46.9%)           | 133 (48.0%)         | 1.022 (0.527–1.980) | 0.850 |
| TT        | 76 (27.5%)            | 72 (26.0%)          | 0.672 (0.309–1.458) | 0.314 |
| CT + TT   | 206 (74.4%)           | 205 (74.0%)         | 0.888 (0.475–1.659) | 0.709 |
Table 2. Cont.

| Genotypes Control (n = 277) | LUAD (n = 277) | AOR (95% CI) | p Value |
|----------------------------|---------------|--------------|---------|
| rs61330082                 |               |              |         |
| GG                         | 69 (24.9%)    | 71 (25.6%)   | 1.000 (reference) |         |
| GA                         | 131 (47.3%)   | 129 (46.6%)  | 0.926 (0.473–1.813) | 0.822 |
| AA                         | 77 (27.8%)    | 77 (27.8%)   | 0.676 (0.313–1.461) | 0.320 |
| GA + AA                    | 208 (75.1%)   | 206 (74.4%)  | 0.829 (0.440–1.562) | 0.563 |
| rs2110385                  |               |              |         |
| GG                         | 225 (81.2%)   | 228 (82.3%)  | 1.000 (reference) |         |
| GT                         | 52 (18.8%)    | 45 (16.2%)   | 0.570 (0.259–1.254) | 0.162 |
| TT                         | 0 (0.0%)      | 4 (1.4%)     | -        | -     |
| GT + TT                    | 52 (18.8%)    | 49 (17.7%)   | 0.637 (0.299–1.356) | 0.242 |
| rs4730153                  |               |              |         |
| GG                         | 223 (80.5%)   | 230 (83.0%)  | 1.000 (reference) |         |
| GA                         | 54 (19.5%)    | 44 (15.9%)   | 0.630 (0.297–1.336) | 0.228 |
| AA                         | 0 (0.0%)      | 3 (1.1%)     | -        | -     |
| GA + AA                    | 54 (19.5%)    | 47 (17.0%)   | 0.667 (0.319–1.393) | 0.281 |

The AORs with 95% CIs were estimated by multiple logistic regression models after controlling for age, gender and cigarette smoking status. Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; LUAD, lung adenocarcinoma.

To examine potential associations between visfatin SNPs and EGFR mutation status, the frequencies of four visfatin SNP genotypes (rs11977021 CT, rs61330082 GA, rs2110385 GT and rs4730153 GA) were compared with EGFR status (Table 3). After adjusting for age, sex, and cigarette smoking status, none of the four visfatin SNPs were associated with a statistically higher prevalence of either EGFR wild-type or EGFR mutation status (Table 3).

Table 3. Distribution frequency of visfatin genotypes and multiple logistic regression analysis of EGFR mutation status in lung adenocarcinoma patients.

| Visfatin Genotypes | EGFR Wild-Type (n = 111) | EGFR Mutation (n = 166) | AOR (95% CI) | p Value |
|--------------------|--------------------------|--------------------------|--------------|---------|
| rs11977021         |                          |                          |              |         |
| CC                 | 30 (27.0%)               | 42 (25.3%)               | 1.000 (reference) |         |
| CT                 | 54 (48.6%)               | 79 (47.6%)               | 1.126 (0.608–2.088) | 0.706 |
| TT                 | 27 (24.4%)               | 45 (27.1%)               | 1.247 (0.613–2.353) | 0.542 |
| CT + TT            | 81 (73.0%)               | 124 (74.7%)              | 1.080 (0.809–1.442) | 0.601 |
| rs61330082         |                          |                          |              |         |
| GG                 | 28 (25.2%)               | 43 (25.9%)               | 1.000 (reference) |         |
| GA                 | 54 (48.6%)               | 75 (45.2%)               | 0.992 (0.531–1.857) | 0.981 |
| AA                 | 29 (26.2%)               | 48 (28.9%)               | 1.116 (0.554–2.250) | 0.759 |
| GA + AA            | 83 (74.8%)               | 123 (74.1%)              | 1.018 (0.760–1.363) | 0.904 |
| rs2110385          |                          |                          |              |         |
| GG                 | 93 (83.8%)               | 135 (81.3%)              | 1.000 (reference) |         |
| GT                 | 17 (15.3%)               | 28 (16.9%)               | 1.065 (0.530–2.140) | 0.859 |
| TT                 | 1 (0.9%)                 | 3 (1.8%)                 | 3.833 (3.370–39.768) | 0.260 |
| GT + TT            | 18 (16.2%)               | 31 (18.7%)               | 1.089 (0.777–1.527) | 0.619 |
Table 3. Cont.

| Visfatin Genotypes | EGFR Wild-Type (n = 111) | EGFR Mutation (n = 166) | AOR (95% CI) | p Value |
|--------------------|--------------------------|-------------------------|--------------|---------|
| rs4730153          |                          |                         |              |         |
| GG                 | 93 (83.8%)               | 137 (82.5%)             | 1.000 (reference) |         |
| GA                 | 17 (15.3%)               | 27 (16.3%)              | 1.027 (0.509–2.072) | 0.941   |
| AA                 | 1 (0.9%)                 | 2 (1.2%)                | 3.241 (0.281–37.379) | 0.346   |
| GA + AA            | 18 (16.2%)               | 29 (17.5%)              | 1.057 (0.751–1.487) | 0.751   |

AORs with 95% CIs were estimated using multiple logistic regression models after controlling for age, sex, and cigarette smoking status. Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; EGFR, epidermal growth factor receptor.

3.3. Associations between Polymorphic Genotypes of visfatin rs11977021 with Clinicopathologic Characteristics and EGFR Status

Clinicopathologic characteristics of LUAD patients stratified by visfatin rs11977021 genotypes are shown in Table 4. In EGFR-mutant patients, LUAD stage and grade did not differ significantly when comparing the CT + TT and CC genotypes. In contrast, among EGFR wild-type patients, the CT + TT genotype was significantly more frequent than the CC genotype in patients with stage III/IV malignancies (81.5% vs. 63.3%, p = 0.045), advanced (T3/T4) tumors (53.1% vs. 26.7%, p = 0.013), and in those with distal metastases (58.0% vs. 33.3%, p = 0.021). The significant difference in tumor status between the CT + TT and CC genotypes in EGFR wild-type patients was also observed in an evaluation of all patients, in which 43.9% with the CT + TT genotype had more advanced (T3/T4) tumors compared with 27.8% with the CC genotype (p = 0.016).

Table 4. Clinicopathologic characteristics of lung adenocarcinoma patients stratified by EGFR status and genotypes at visfatin rs11977021.

| Variable | All (N = 277) | EGFR Wild-Type (N = 111) | EGFR Mutation (N = 166) |
|----------|---------------|--------------------------|--------------------------|
|          | CC (n = 72)   | CT + TT (n = 205)        | p Value                  | CC (n = 30)   | CT + TT (n = 81) | p Value                  |
|          |               |                          |                          |               |                  |
| Stage    |               |                          | p = 0.475                | p = 0.045 b   |
| I/II     | 21 (29.2%)    | 51 (24.9%)               | 11 (36.7%)               | 15 (18.5%)    | 10 (23.8%)       | 36 (29.0%)               | 0.513 |
| III/IV   | 51 (70.8%)    | 154 (75.1%)              | 19 (63.3%)               | 66 (81.5%)    | 32 (76.2%)       | 88 (71.0%)               |         |
| Tumor status |               |                          |                          |               |                  |
| T1/T2    | 52 (72.2%)    | 115 (56.1%)              | p = 0.016 a               | 22 (73.3%)    | 38 (46.9%)       | p = 0.013 c               | 30 (71.4%) | 77 (62.1%) | 0.275 |
| T3/T4    | 20 (27.8%)    | 90 (43.9%)               | 8 (26.7%)                | 43 (53.1%)    | 12 (26.6%)       | 47 (37.9%)               |         |
| Lymph node status |               |                          |                          |               |                  |
| Negative | 22 (30.6%)    | 61 (29.8%)               | p = 0.899                | 10 (33.3%)    | 19 (23.5%)       | p = 0.293                | 12 (28.6%) | 42 (33.3%) | 0.526 |
| Positive | 50 (69.4%)    | 144 (70.2%)              |                          | 20 (66.7%)    | 62 (76.5%)       |                          | 30 (71.4%) | 82 (66.1%) |         |
| Distant metastases |               |                          |                          |               |                  |
| Negative | 41 (56.9%)    | 91 (44.4%)               | p = 0.067                | 20 (66.7%)    | 34 (42.0%)       | p = 0.021 d               | 21 (50.0%) | 57 (46.0%) | 0.651 |
| Positive | 31 (43.1%)    | 114 (55.6%)              |                          | 10 (33.3%)    | 47 (58.0%)       |                          | 21 (50.0%) | 67 (54.0%) |         |
| Cell differentiation |               |                          |                          |               |                  |
| Well/Moderate | 65 (90.3%)    | 179 (87.3%)              | p = 0.505                | 25 (83.3%)    | 63 (77.8%)       | p = 0.521                | 40 (95.2%) | 116 (93.5%) | 0.691 |
| Poor     | 7 (9.7%)      | 26 (12.7%)               |                          | 5 (16.7%)     | 18 (22.2%)       |                          | 2 (4.8%)  | 8 (6.5%)  |         |

a OR (95% CI): 2.035 (1.134–3.652); b OR (95% CI): 2.547 (1.005–6.459); c OR (95% CI): 3.112 (1.241–7.804); d OR (95% CI): 2.765 (1.149–6.652). Abbreviation: EGFR, epidermal growth factor receptor.

3.4. Associations between Polymorphic Genotypes of visfatin rs61330082, Clinicopathologic Characteristics and EGFR Status

Clinicopathologic characteristics of LUAD patients stratified by visfatin rs61330082 genotypes are shown in Table 5. In EGFR-mutant patients, LUAD stage and grade did not differ significantly in a comparison of the GA + AA genotype with the GG genotype. Among EGFR wild-type patients, the GA + AA genotype was significantly more common than
the GG genotype in patients with advanced (T3/T4) tumors (53.0% vs. 25.0%, \( p = 0.010 \)) and in those with distal metastases (57.8% vs. 32.1%, \( p = 0.019 \)). The significant difference in tumor status between the GA + AA and GG genotypes with EGFR wild-type status was also observed when all patients were analyzed, as 43.7% of those with the GA + AA genotype had advanced (T3/T4) tumors compared with 28.2% with the GG genotype (\( p = 0.021 \)). There was no association between polymorphic genotypes of visfatin rs2110385 or rs4730153 with clinicopathological characteristics and EGFR status in LUAD patients (Tables S3 and S4).

**Table 5.** Clinicopathologic characteristics of lung adenocarcinoma patients stratified by EGFR status and genotypes at visfatin rs61330082.

| Variable                  | All (N = 277) | EGR Wild-Type (N = 111) | EGR Mutation (N = 166) |
|---------------------------|--------------|-------------------------|------------------------|
|                           | GG (n = 71)  | GA + AA (n = 206)       | GG (n = 28)            | GA + AA (n = 83) |
|                           |              | \( p = 0.628 \)         | \( p = 0.076 \)        | \( p = 0.448 \) |
|                           |              | 20 (28.2%)              | 10 (35.7%)             | 10 (23.3%)       |
| Stage                     |              | 52 (25.2%)              | 16 (19.3%)             | 36 (29.3%)       |
| I/II                      |              | 154 (74.8%)             | 67 (80.7%)             | 87 (70.7%)       |
|                           |              | \( p = 0.021 \)         | \( p = 0.001 \)        | \( p = 0.398 \) |
|                           |              | 51 (71.8%)              | 21 (75.0%)             | 30 (69.8%)       |
|                           |              | 116 (56.3%)             | 39 (47.0%)             | 77 (62.6%)       |
| Tumor status              |              | 90 (43.7%)              | 44 (53.0%)             | 46 (37.4%)       |
| T1/T2                     | 20 (28.2%)   | 7 (25.0%)               | 13 (30.2%)             | \( p = 0.452 \) |
|                           |              | \( p = 0.093 \)         | \( p = 0.402 \)        | \( p = 0.778 \) |
|                           | 50 (70.4%)   | 9 (32.1%)               | 20 (24.1%)             | 42 (34.1%)       |
|                           |              | 144 (69.9%)             | 63 (75.9%)             | 81 (65.9%)       |
| Lymph node status         |              |                         |                       | \( p = 0.452 \) |
| Negative                  | 21 (29.6%)   | 9 (32.1%)               | 20 (24.1%)             | 42 (34.1%)       |
|                           |              | 62 (30.1%)              | 63 (75.9%)             | 81 (65.9%)       |
|                           | 50 (70.4%)   | 19 (67.9%)              | 31 (72.1%)             | \( p = 0.452 \) |
| Positive                  | 144 (69.9%)  | 63 (75.9%)              | 81 (65.9%)             | \( p = 0.452 \) |
| Distant metastases        |              |                         |                       | \( p = 0.778 \) |
| Negative                  | 40 (56.3%)   | 19 (67.9%)              | 21 (48.8%)             | 57 (46.3%)       |
|                           |              | 92 (44.7%)              | 35 (42.2%)             | 57 (46.3%)       |
|                           | 114 (55.3%)  | 48 (57.8%)              | 22 (51.2%)             | \( p = 0.778 \) |
|                           | \( p = 0.089 \) | 35 (42.2%)     | 48 (57.8%)             | 22 (51.2%)       |
| Positive                  | 31 (43.7%)   | 48 (57.8%)              | 22 (51.2%)             | \( p = 0.778 \) |
|                           | 114 (55.3%)  | 48 (57.8%)              | 22 (51.2%)             | \( p = 0.778 \) |
| Cell differentiation      |              |                         |                       | \( p = 0.778 \) |
| Well/Moderate             | 65 (91.5%)   | 25 (89.3%)              | 40 (93.0%)             | 116 (94.3%)      |
|                           | 179 (86.9%)  | 63 (75.9%)              | 116 (94.3%)            | \( p = 0.760 \) |
|                           | 27 (13.1%)   | 20 (24.1%)              | 3 (7.0%)               | \( p = 0.760 \) |
| Poor                      | 6 (8.5%)     | 3 (10.7%)               | 3 (7.0%)               | \( p = 0.760 \) |

\( ^a \text{OR (95\% CI): 1.978 (1.101–3.554)}; ^b \text{OR (95\% CI): 3.385 (1.299–8.821)}; ^c \text{OR (95\% CI): 2.895 (1.171–7.156).} \) Abbreviation: EGFR, epidermal growth factor receptor.

4. Discussion

Our observation that EGFR mutations are more common in females than males and in never-smokers than in ever-smokers among patients with LUAD is consistent with previous reports [6,29]. We also found that EGFR wild-type disease was more likely to exhibit poor cell differentiation and lower rates of well or moderate cell differentiation compared to EGFR-mutant disease, which is consistent with previous reports [12,13].

In this study, the four visfatin SNP rs11977021, rs61330082, rs2110385 and rs4730153, had no association with EGFR wild-type or EGFR-mutant status. However, when analyzing visfatin rs11977021 and rs61330082 genotypes in relation to clinicopathologic characteristics, the rates of the rs11977021 CT + TT and rs61330082 GA + AA genotypes were higher than those of the CC and GG genotypes, respectively, in EGFR wild-type patients with advanced (T3/T4) tumors and those with distal metastases. Among EGFR wild-type patients, having the CT + TT genotypes at rs11977021 was associated with more severe (stage III/IV) malignancy compared with having the CC genotype at rs11977021. In an analysis of the total study population, advanced (T3/T4) tumors were found in individuals with the SNP rs11977021 carrying the CT + TT genotypes compared with those carrying the CC genotype and in those with the SNP rs61330082 and the GA + AA genotypes compared with the GG genotype.

The rs11977021 and rs61330082 SNPs are located at the promoter region of visfatin and have been documented to affect the transcription activity of visfatin [30]. Among the four studied visfatin SNPs, rs11977021 was predicted to be a potential methylation site after analysis using NmSEER V2.0 online software (http://www.manut.net/nmseer-v2/,
Further studies are needed to clarify whether specific genotypes at this SNP affect visfatin’s methylation status and expression level.

In our study, neither visfatin SNPs rs4730153 nor rs2110385 were associated with particular clinicopathologic characteristics in LUAD. However, our previous study reported that the SNP rs4730153 GA genotype was associated with lymph node metastasis in OSCC betel nut chewers, whereas the SNPs rs2110385 and rs61330082 had no relation to OSCC [23].

The visfatin SNP rs11977021 has been investigated in other cancers. Our previous investigation found that having the visfatin SNP rs11977021 and the CT + TT genotypes was associated with a lower risk of developing OSCC compared with those with the CC genotype at the same SNP [23]. In contrast, in another study involving patients with hepatitis B virus (HBV) infection, there was no significant association between visfatin rs11977021 and the risk of developing HBV-related hepatocellular carcinoma (HBV-HCC) [24].

In this study, our analysis of the G/A alleles of visfatin rs61330082 SNP revealed that the GA + AA genotypes was more likely than the GG to be associated with severe disease characteristics in LUAD. The GA + AA and GG genotypes in our study are equivalent to the CT + TT and CC genotypes, respectively, described in the studies referenced in this paragraph since they refer to the genotypes on the minus (antisense) strand [6,19,24,25,32]. Previous analyses of visfatin SNP rs61330082 have reported that it is associated with an increased risk of NSCLC, esophageal squamous cell cancer (ESCC), bladder cancer, and HCC [6,19,24,25,32]. In patients with NSCLC, the CT genotype, TT genotype and T allele of visfatin SNP rs61330082 apparently reduced the risk of NSCLC pathogenesis, whereas the CC genotype appeared to increase the risk [19]. Similarly, the CC genotype and C allele of visfatin SNP rs61330082 were associated with increased risk of ESCC [25] and bladder cancer, especially in smokers [32].

By contrast, in HBV-HCC, the TT genotype of visfatin rs61330082 was associated with a higher risk of HBV-HCC than the CC and CC + TT genotypes, but only in patients of Zhuang but not Han ethnicity [24]. Thus, it appears that visfatin rs61330082 polymorphisms in cancer development vary among different tumor tissues. Further studies are required to clarify the role of visfatin rs61330082 polymorphisms in LUAD tumorigenesis and progression.

Elevated visfatin levels have been detected in tumor and plasma samples in many cancers, including NSCLC [17,19]. One potential mechanism for increased visfatin levels may be due to specific genotypes in rs11977021 and rs61330082 resulting in increased transcription activity, although this has only been reported in obese children [30,33]. Visfatin is critical in NSCLC, due to its ability to increase intracellular ATP and NAD$^+$ levels [16]. ATP is required to enhance the activity of receptor tyrosine kinases such as EGFR, which are in turn involved in signaling pathways needed for the survival and growth of NSCLC cells, while NAD$^+$ is a substrate for enzymes such as poly (ADP-ribose) polymerase-1 and sirtuin that contribute to apoptosis resistance and tumor cell survival [16].

Visfatin (NAMPT) plays a pivotal role in LUAD cell survival, with NAMPT inhibition via NAMPT-small interfering RNA (siRNA) or the NAMPT inhibitor FK866 reducing the
proliferation of three LUAD cell lines in one study [16], while FK866 also reduced intracellular ATP levels, dephosphorylated EGFR signal proteins and promoted apoptosis in the H1975 cell line [16]. Visfatin inhibitors work because cancer cells are more sensitive to visfatin inhibition than normal cells due to their reliance on NAD⁺ and the cancer cells are more dependent on NAD-mediated processes [21]. Normal cells, however, can synthesize NAD through an alternative pathway, i.e., nicotinic acid phosphoribosyltransferase (NaPRTase) and thereby protect themselves from visfatin inhibition [21].

5. Conclusions

Obesity is a risk factor for many cancers [34]. Although this may not appear to be the case in LUAD, which is inversely associated with high body mass index, a meta-analysis of prospective cohort studies that examined the association between measures of abdominal obesity and risk of lung cancer found that in lung cancer generally, abdominal obesity is a better predictor of malignancy than general obesity [35]. Furthermore, since visfatin expression is particularly enriched in visceral fat, abdominal obesity could be a possible source of elevated serum visfatin levels in NSCLC patients [19,36]. Indeed, higher visfatin levels have been found in obese individuals compared with those in nonobese controls [37,38]. Thus, it would appear that while NSCLC cells likely need to produce visfatin to survive, any extra visfatin secreted from adipose tissue would only serve to improve the tumor microenvironment.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijerph192215172/s1, Figure S1: Representative TaqMan assay for visfatin rs11977021 genotyping; Figure S2: Representative TaqMan assay for visfatin rs61330082 genotyping; Figure S3: Representative TaqMan assay for visfatin rs2110385 genotyping; Figure S4: Representative TaqMan assay for visfatin rs4730153 genotyping; Table S1: Demographics and clinical characteristics of 277 controls and 277 patients with lung adenocarcinoma; Table S2: The context sequences of the four visfatin SNPs in this study; Table S3: Clinicopathologic characteristics of lung adenocarcinoma patients stratified by EGFR status and genotypes at visfatin rs2110385; Table S4: Clinicopathologic characteristics of lung adenocarcinoma patients stratified by EGFR status and genotypes at visfatin rs4730153; Table S5: ALFA allele frequencies of visfatin SNPs.

Author Contributions: S.-F.Y. and C.-H.T. conceived and designed the project. C.-H.T. supervised the project. The data curation, validation and methodology were performed by S.L.-Y.C., P.-J.Y., Y.-Y.L. and Y.-J.J. Reagents, materials, and analysis tools were supplied by S.L.-Y.C., P.-I.L. and C.-L.H. The manuscript was written by S.L.-Y.C. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available on request from the corresponding authors.

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