Association between squamous cell carcinoma antigen level and EGFR mutation status in Chinese lung adenocarcinoma patients

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Abstract
Background: To investigate the association between squamous cell carcinoma antigen (SCCAg) level and epidermal growth factor receptor (EGFR) mutation status in Chinese lung adenocarcinoma patients.

Methods: We retrospectively analyzed 293 patients with lung adenocarcinoma, divided into EGFR mutant group (n = 178) and EGFR wild-type group (n = 115). The general data and laboratory parameters of the two groups were compared. We used univariable and multivariable logistic regression to analyze the association between SCCAg level and EGFR mutation. Generalized additive model was used for curve fitting, and a hierarchical binary logistic regression model was used for interaction analysis.

Results: Squamous cell carcinoma antigen level in the EGFR wild-type group was significantly higher than that in the mutant group (p < 0.001). After adjusting for confounding factors, we found that elevated SCCAg was associated with a lower probability of EGFR mutation, with an OR of 0.717 (95% CI: 0.543–0.947, p = 0.019). For the tripartite SCCAg groups, the increasing trend of SCCAg was significantly associated with the decreasing probability of EGFR mutation (p for trend = 0.015), especially for Tertile 3 versus Tertile 1 (OR = 0.505; 95% CI: 0.258–0.986; p = 0.045). Curve fitting showed that there was an approximate linear negative relationship between continuous SCCAg and EGFR mutation probability (p = 0.020), which was first flattened and then decreased (p < 0.001). The association between the two was consistent among different subgroups, suggesting no interaction (all p > 0.05).

Conclusion: There is a negative association between SCCAg level and EGFR mutation probability in Chinese lung adenocarcinoma patients.

Keywords
epidermal growth factor receptor, lung adenocarcinoma, risk factor, squamous cell carcinoma antigen
1 | INTRODUCTION

Lung cancer is the most common type of cancer in the world. One-third of global lung cancer cases are in China, ranking first in morbidity and mortality. Non-small cell lung cancer (NSCLC) is the predominant type of lung cancer, accounting for approximately 80%–85%, of which adenocarcinoma is one of the most common histopathological types. In recent years, the treatment methods for lung cancer have been continuously updated and developed. For patients with inoperable advanced NSCLC, the current treatment mode is shifting from simple cytotoxic radiotherapy and chemotherapy to individualized, targeted therapy. Compared with traditional chemotherapy, targeted therapy has a longer survival time and fewer side effects, and the life quality of patients is higher, which provides a new treatment direction for patients with advanced NSCLC.

In the past decade, targeted therapy for lung cancer has made great progress, especially the epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), one of the primarily targeted drugs used in NSCLC patients. EGFR-TKI has been shown to prolong progression-free survival and overall survival of NSCLC patients, and its efficacy and prognosis are closely related to the mutation status of the EGFR gene. Therefore, identifying EGFR mutation status is particularly important for TKI therapy in NSCLC patients. However, in reality, only a few patients are tested for EGFR gene mutation, which may be due to the difficulty in obtaining tumor tissue samples, the high cost of EGFR mutation detection, and the limited detection technology. Therefore, a simple, non-invasive and low-cost detection method that can accurately predict EGFR mutation status can guide the individualized management and treatment of NSCLC patients.

Previous studies have shown that clinicopathological features, liquid biopsy, serum tumor markers, imaging features, and other indicators have potential correlations with EGFR gene mutations. SCCAg, a serum tumor marker, is mainly present in the cytoplasm of lung, uterine, and esophageal squamous cell carcinomas, and high levels of SCCAg are often associated with poorly differentiated and advanced metastatic squamous cell carcinomas. Our study aimed to investigate the relationship between SCCAg level and EGFR mutation status in patients with lung adenocarcinoma, hoping to guide the targeted therapy in patients with lung adenocarcinoma.

2 | MATERIALS AND METHODS

2.1 | Clinical data

We retrospectively analyzed lung cancer patients who underwent surgical resection or needle biopsy in the Third Affiliated Hospital of Soochow University from January 2018 to December 2020. The inclusion criteria were: (1) Lung adenocarcinoma was confirmed by surgery or biopsy pathology, and the pathological classification was based on the lung adenocarcinoma classification criteria published by the International Association for the Study of Lung Cancer (IASLC), American Thoracic Society (ATS), and European Respiratory Society (ERS); (2) there was a clear EGFR test result; (3) patients had no history of other tumors, severe liver disease or diabetes. The exclusion criteria were: (1) other pathological subtypes; (2) no chest thin-slice CT results; (3) poor CT image quality or difficult-to-measure lesions; (4) missing SCCAg results. The general information of enrolled patients, including age, sex, smoking status, results of thin-section CT imaging features, and serum tumor markers, were recorded. This study followed the principles of the Declaration of Helsinki and was approved by the Ethics Committee of our hospital [ethics number: (2019) JD 79]. The informed consent was not required because the patients were anonymous. The research flow chart is shown in Figure 1.
2.2 | Laboratory test results

2.2.1 | EGFR mutation detection

The EGFR gene mutation detection kit from Shanghai Yuanqi company was used with PCR fluorescent probe method to detect the mutations on EGFR gene Exon18 (G719C, G719S), Exon19 (2235-2249del, 2236-2250del, 2236-2253del, 2239-2253del, 2239-2256del, 2240-2251del, 2240-2254del, 2240-2257del, 2237-2255>G, 2238-2248>G, 2237-2252>GCA, 2239-2251>C, 2254-2277del, 2238-2255del, 2240-2248del, 2239-2259del), Exon20 (V769_D770insASV, D770_N771insG, H773_V774insH), and Exon21 (L858R, L861Q).

Detection method

The DNA was extracted from samples such as paraffin-embedded pathological tissues or sections of patients and amplified on ABI 7300 fluorescence PCR detector. The amplification conditions were: 42°C, 5 min; 94°C, 3 min; (94°C, 15 s; 60°C, 60 s) for 40 cycles; the reaction volume was 25 μl; the fluorescence signal was collected at 60°C in the second step of the PCR cycle; the detection channel was FAM-TAMRA, and the reference fluorescence was set to none. The computer automatically processed and analyzed the data.

Interpretation of results

The interpretation of the results refers to the interpretation principles of the detection kit.

1. Negative control validity judgment

For EGFR gene (exon18, exon20, and exon21), internal reference gene: C_T (Cycle threshold) value ≥38 or display "Undet" is judged as valid; for EGFR gene (exon19): C_T value ≥38 or display "Undet"; or the C_T value difference between related gene and internal reference gene ≥7 is judged as valid.

2. Positive control validity judgment

For EGFR gene (exon18, exon20, and exon21): C_T value <36 is judged as valid; for EGFR gene (exon19), internal reference gene: C_T value <36, and the C_T value difference between related gene and internal reference gene ≤1 is judged as valid.

3. Judgment of PCR results

a. The internal reference gene C_T value <38

For EGFR gene (exon18, exon20, and exon21)

The target gene C_T value <38 is judged as a mutation in the detected gene; (2) The target gene C_T value ≥38 or display "Undet" is judged as no mutations or mutations below the minimum detection limit.

For EGFR gene (exon19):

The target gene C_T value <38, and ΔC_T value (C_T value difference between target gene and internal reference gene) ≤1 is judged as a mutation in the detected gene; (2) The target gene C_T value <38, and ΔC_T value between 1 and 7, is judged as a small amount of EGFR (exon19) mutation. It is suggested to deal with it according to the clinical situation; (3) The target gene C_T value ≥38 or display "Undet"; or target gene C_T value <38, and ΔC_T value ≥7 is judged as no mutations or mutations below the minimum detection limit.

b. The internal reference gene C_T value ≥38

For EGFR gene (exon18, exon20, and exon21):

The target gene C_T value <38 is judged as a mutation in the detected gene; (2) The target gene C_T value ≥38 or display "Undet" is determined that the sampling amount needs to be increased and re-extracted for detection, to avoid missed detection due to insufficient DNA addition.

For EGFR gene (exon19):

The target gene C_T value <38, and ΔC_T value ≤1 is judged as a mutation in the detected gene; (2) The target gene C_T value <38, and ΔC_T value between 1 and 7, is judged as a small amount of EGFR (exon19) mutation. It is suggested to deal with it according to the clinical situation; (3) The target gene C_T value ≥38 or display "Undet"; or target gene C_T value <38, and ΔC_T value ≥7 is determined that the sampling amount needs to be increased and re-extracted for detection, to avoid missed detection due to insufficient DNA addition.

Quality control

During the test, the quality of DNA used for detection is very important. The test should be carried out as soon as possible after DNA extraction. Fluorescence quantitative PCR (FQ-PCR) is a high-sensitivity experiment that should be operated in strict accordance with the operating specifications of PCR laboratory and the safety specifications of biological products. At the same time, it must pay attention to anti-contamination and strictly distinguish the use of positive quality control materials and reaction reagents to avoid false positives.

2.3 | Image analysis

The type (solid, sub-solid), location (upper, lower lobe of left lung, upper, middle, and lower lobe of right lung), shape (circular/oval, polygonal/irregular), edge (lobulated, spicule-like), bronchial sign, vacuole sign, pleural indentation sign, vessel convergence sign, long and short diameters of lesions (measured at the largest cross-section of the tumor, long and short diameters were perpendicular to each other) on thin-slice CT were recorded. All parameters were observed and recorded by two radiologists with more than 10 years of experience without knowing the results of EGFR testing.

2.4 | Detection of serum tumor markers

The venous blood was collected from patients, and the serum levels of carcinoembryonic antigen (CEA, reference range: 0-5 ng/ml), cytokeratin soluble fragment 19 (CYFRA21-1, reference range: 0-3.3 ng/ml), neuron-specific enolase (NSE, reference range:
0-17 ng/ml, and SCCAg (reference range: 0–2.7 ng/ml) were measured by electrochemiluminescence immunoassay analyzer Cobas 8000 e801 (Indianapolis) invented and registered by Roche company. The performance indicators of all serum tumor markers, such as minimum detection limit, inter-assay precision, and intra-assay precision, are shown in Table S1.

2.5 | Statistical analysis

Statistical analysis was performed using R software (version 3.4.3: http://www.R-project.org/). Continuous variables were expressed as mean (standard deviation) (normal distribution) or Median (Q1–Q3) (skewed distribution); categorical variables were expressed as frequency or rate (%), $\chi^2$ test (categorical variable), T test (normal distribution), or Mann–Whitney U test (skewed distribution) were used to compare the differences in general data and laboratory parameters between different EGFR mutation groups (binary variables).

We used univariable and multivariable logistic regression methods to examine the correlation between SCCAg levels and EGFR mutation to construct three different models, including unadjusted, preliminarily adjusted, and fully adjusted models. In multivariable regression analysis, when a factor was introduced into the basic model or excluded from the complete model if the regression coefficient of SCCAg level changed by more than 10% or the factor was significantly associated with EGFR mutation ($p < 0.1$), then it was included into the final model as a potential confounding factor. To test the robustness of the results, we performed a sensitivity analysis, transforming SCCAg levels into categorical variables by tripartition and calculating $p$ values for trend.

After fully adjusting for covariates, a generalized additive model (GAM) was used for curve fitting, and hierarchical binary logistic regression models were used to assess whether there was an interaction between SCCAg and EGFR mutation in different subgroups. The effect size with a 95% confidence interval was recorded. All statistical tests were two-sided, and $p < 0.05$ was considered statistically significant. No data imputation was used for missing data (covariates).

3 | RESULTS

Finally, 293 patients with lung adenocarcinoma were enrolled in this study, including 148 females and 145 males, with an average age of 64.2 ± 9.4 years (38–84 years old). There were 110 (37.5%) smokers and 93 (31.7%) cases with subsolid nodules. The clinical stages included: 129 cases (44.0%) of stage I, 11 cases (3.8%) of stage II, 49 cases (16.7%) of stage III, and 104 cases (35.5%) of stage IV. After pathological confirmation by surgery, puncture, or bronchoscopy, 115 cases (39.3%) were EGFR wild-type, and 178 cases (60.8%) were EGFR mutant (1 case on exon 18, 73 cases on exon 19, 8 cases on exon 20, 90 cases on exon 21, 1 case on exon 19 and 20, 2 cases on exon 19 and 21, and 3 cases on unknown exon).

3.1 | Comparison of general data, morphological characteristics, and laboratory parameters between EGFR mutant group and wild-type group

The results showed that the proportions of males, smokers, and solid nodules in the wild-type group were significantly higher than those in the mutant group (67.0% vs. 38.2%, 55.7% vs. 25.8%, 77.4% vs. 62.4%, respectively; all $p < 0.05$). The proportions of bronchial sign, pleural indentation sign, and vessel convergence sign in the mutant group were significantly higher than those in the wild-type group (57.3% vs. 44.4%, 71.4% vs. 48.7%, 60.7% vs. 47.8%, respectively; all $p < 0.05$). The clinical stage and tumor long diameter of the wild-type group were significantly higher than those of the mutant group (all $p < 0.01$), and there was no significant difference in tumor short diameter ($p = 0.070$). For tumor indicators, the level of CEA in the wild-type group was higher than that in the mutant group, but the difference was not significant ($p = 0.068$), while the level of SCCAg in the wild-type group was significantly higher than that in the mutant group ($p < 0.001$) (Table 1).

3.2 | Multivariable regression analysis for the association between SCCAg and EGFR mutation

Table 2 shows the univariable and multivariable logistic regression analyses for continuous SCCAg and tripartite SCCAg. Unadjusted covariates were equivalent to univariable logistic regression analysis. Preliminarily adjusted covariates included age, sex, and smoking history. Fully adjusted covariates included age, sex, smoking history, nodule type, bronchial sign, pleural indentation sign, vessel convergence sign, tumor short diameter, and clinical stage (variables excluded by a variance inflation factor (VIF ≥5): tumor long diameter). For continuous SCCAg, the increase of SCCAg was associated with decreased probability of EGFR mutation in unadjusted, preliminarily adjusted, and fully adjusted regression equations and the ORs were 0.596, 0.702, and 0.717, respectively ($p < 0.05$ for all).

For tripartite SCCAg, the increasing trend of SCCAg was significantly associated with decreased probability of EGFR mutation in the unadjusted, preliminarily adjusted, and fully adjusted regression equations ($p$ for trend $<0.05$ for all), especially for Tertile 3 versus Tertile 1 in unadjusted and fully adjusted covariates (OR were 0.342 and 0.505, $p < 0.05$ for both).

3.3 | Smooth curve fitting between SCCAg and EGFR mutation probability

Generalized additive model test results showed that, after the adjustment for age, sex, smoking history, nodule type, bronchial sign, pleural indentation sign, vessel convergence sign, tumor short diameter, and clinical stage, there was an approximately linear relationship between continuous SCCAg and EGFR mutation probability (degree of freedom = 1.023, $\chi^2 = 5.648$, $p = 0.020$); with
the increase of SCCAg, the probability of EGFR mutation was significantly decreased, and the OR was 0.717 (95% CI: 0.543–0.947; \( p = 0.019 \)) (Figure 2A). If using tripartite SCCAg grouping [96 cases in Tertile 1 (0.29–0.67), 89 cases in Tertile 2 (0.68–0.99), and 108 cases in Tertile 3 (1.00–11.50)], the relationship between different levels of SCCAg and EGFR mutation probability showed a trend of first flattening and then decreasing [70.8% (68/96), 68.5% (61/89), and 45.4% (49/108), \( p < 0.001 \)]; after fully adjusted for covariates, the probability of EGFR mutation was 70.8% (95% CI: 52.9%–84.0%), 74.5% (95% CI: 56.6%–86.8%) and 55.1% (95% CI: 35.4%–73.2%) with the increase of SCCAg level (Figure 2B).

### 3.4 Interaction analysis

After adjusting for age, sex, smoking history, nodule type, bronchial sign, pleural indentation sign, vessel convergence sign, tumor short diameter, and clinical stage, we analyzed the relationship between

| EGF | Wild-type group | Mutant group | \( p \)-Value |
|-----|----------------|--------------|--------------|
| N   | 115            | 178          | 0.475        |
| Age (years) | 64.9 (9.5) | 63.9 (9.4) | 0.475 |
| Sex  |                |              |              |
| Female | 38 (33.0%) | 110 (61.8%) | <0.001 |
| Male   | 77 (67.0%) | 68 (38.2%) |              |
| Smoking history | 64 (55.7%) | 46 (25.8%) | <0.001 |
| Nodule type |            |              |              |
| Solid  | 89 (77.4%) | 111 (62.4%) | 0.007 |
| Subsolid | 26 (22.6%) | 67 (37.6%) |              |
| Location |            |              |              |
| Upper right lung | 34 (29.6%) | 60 (33.7%) | 0.732 |
| Middle right lung | 4 (3.5%) | 11 (6.2%) |              |
| Lower right lung | 24 (20.9%) | 36 (20.2%) |              |
| Upper left lung | 33 (28.7%) | 44 (24.7%) |              |
| Lower left lung | 20 (17.4%) | 27 (15.2%) |              |
| Shape |                |              |              |
| Round/oval | 66 (57.4%) | 92 (51.7%) | 0.339 |
| Polygonal/irregular | 49 (42.6%) | 86 (48.3%) |              |
| Lobulation sign | 96 (83.5%) | 155 (87.1%) | 0.390 |
| Spicule sign | 59 (51.3%) | 98 (55.1%) | 0.529 |
| Bronchial sign | 51 (44.4%) | 102 (57.3%) | 0.030 |
| Vacuole sign | 18 (15.7%) | 23 (12.9%) | 0.511 |
| Pleural indentation sign | 56 (48.7%) | 127 (71.4%) | <0.001 |
| Vessel convergence sign | 55 (47.8%) | 108 (60.7%) | 0.031 |
| Clinical stage |            |              | 0.006        |
| I     | 36 (32.2%) | 92 (51.7%) |              |
| II    | 8 (7.0%)  | 3 (1.7%)   |              |
| III   | 22 (19.1%) | 27 (15.2%) |              |
| IV    | 48 (41.7%) | 56 (31.5%) |              |
| Tumor long diameter (mm) | 32.0 (20.7–44.9) | 25.7 (19.9–37.3) | 0.013 |
| Tumor short diameter (mm) | 20.8 (15.3–30.1) | 19.0 (14.1–27.3) | 0.070 |
| CEA (ng/ml) | 4.59 (2.46–15.95) | 3.38 (1.58–11.57) | 0.068 |
| CYFRA21-1 (ng/ml) | 3.51 (2.40–5.59) | 3.26 (2.10–5.34) | 0.410 |
| NSE (ng/ml) | 14.38 (11.54–20.28) | 14.51 (12.03–19.63) | 0.615 |
| SCCAg (ng/ml) | 1.00 (0.69–1.50) | 0.78 (0.54–1.00) | <0.001 |

Note: Results in the table: Mean (SD) Median (Q1–Q3) / N (%). \( \chi^2 \) test was used for categorical variables; \( T \) test for continuous variables with normal distribution; Mann–Whitney U test for continuous variables with skewed distribution; \( p < 0.05 \) was considered statistically significant.

Abbreviations: EGF, epidermal growth factor receptor; CEA, carcinoembryonic antigen; CYFRA21-1, cytokeratin soluble fragment 19; NSE, neuron-specific enolase; SCCAg, squamous cell carcinoma antigen.
SCCAg and EGFR mutation in different subgroups (including shape, lobulation sign, spicule sign, vacuolar sign, tripartite tumor long diameter, tripartite CEA level, tripartite CYFRA21-1 level, tripartite NSE level) (Figure 3). The results showed that none of the above subgroups significantly changed the association between SCCAg and EGFR mutation (all \( p > 0.05 \)), suggesting no interaction.

### DISCUSSION

In recent years, targeted therapy for lung cancer patients has drawn extensive attention, especially the EGFR-TKI therapy, which has shown significant efficacy in NSCLC patients.\(^6\)\(^,\)\(^19\)\(^,\)\(^20\) Therefore, it is particularly important to predict EGFR mutation status. However, genetic testing is not feasible in many cases.\(^13\) Studies have shown that serum tumor markers have a certain value in predicting EGFR mutation status,\(^21\)\(^,\)\(^22\) but there is still controversy. After fully adjusting for confounding factors, our study found an approximate linear negative correlation between SCCAg and the probability of EGFR mutation in patients with lung adenocarcinoma; also, with the increase of tripartite SCCAg levels, the likelihood of EGFR mutation decreased significantly.

Our results are consistent with many previous studies,\(^21,23–25\) which found that EGFR mutations were more frequent in females and non-smokers. It has been reported that different histological types of NSCLC have different EGFR mutation rates, and lung adenocarcinoma patients are more prone to have EGFR mutations\(^21,26\); moreover, the Asian population has the highest EGFR mutation frequency (51.4%).\(^6\) In this study, the EGFR mutation rate of lung adenocarcinoma patients was 60.8%, and the mutant group was

| Exposure | Unadjusted | Adjust I | Adjust II |
|----------|------------|----------|-----------|
| SCCAg    | OR (95% CI) p value | OR (95% CI) p value | OR (95% CI) p value |
| SCCAg Tertile | | | |
| Tertile 1 (0.29–0.67) n = 96 | 1.0 | 1.0 | 1.0 |
| Tertile 2 (0.68–0.99) n = 89 | 0.897 (0.479, 1.680) 0.734 | 1.216 (0.624, 2.370) 0.566 | 1.203 (0.601, 2.407) 0.602 |
| Tertile 3 (1.00–11.50) n = 108 | 0.342 (0.191, 0.611) <0.001 | 0.540 (0.284, 1.027) 0.060 | 0.505 (0.258, 0.986) 0.045 |
| \( p \) for trend | <0.001 | 0.020 | 0.015 |

Note: Results in the table: OR (95% CI) p-value. Univariable and multivariable logistic regression methods were used to examine the association between SCCAg levels and EGFR mutations, and three different models were constructed. SCCAg levels were further transformed into categorical variables by tripartition and calculating \( p \) for trend; \( p < 0.05 \) was considered statistically significant. Unadjusted model adjusted for: None. Adjust I model adjust for: age; sex; smoking history. Adjust II model adjust for: age; sex; smoking history; nodule type; bronchial sign; pleural indentation sign; vessel convergence sign; tumor short diameter; clinical stage.

Abbreviation: SCCAg, squamous cell carcinoma antigen.
more likely to have imaging signs such as bronchial sign, pleural indentation sign, and vessel convergence sign. A meta-analysis also pointed out that some CT imaging features were risk factors for EGFR mutation in NSCLC patients. In clinical practice, the detection rate of EGFR mutation is much lower than expected due to the lack of tumor specimens, poor quality of specimens, and economic reasons. Therefore, we need to find a simple, non-invasive, and convenient alternative method. However, only relying on these clinical factors to determine the mutational status of EGFR is not enough.

Serum tumor markers play an important role in cancer diagnosis, treatment, and follow-up monitoring. CEA, CYFRA21-1, NSE, and SCCAg are used clinically for the diagnosis and prognosis evaluation of lung cancer, but whether they are related to EGFR mutation status is still unclear. Cai et al. believed that the level of CEA was an independent factor for predicting EGFR gene mutation, and the incidence of EGFR gene mutation gradually increased with the increase of serum CEA level. However, other studies did not find any correlation between the two. In this study, the CEA level in the wild-type group was higher than that in the mutant group, but the difference was not significant, which may be related to the inclusion of larger and more diverse lung adenocarcinoma samples. In addition, this study found no associations between CYFRA21-1 or NSE and EGFR mutation. It is generally believed that CYFRA21-1 is more useful for squamous cell carcinoma, while NSE is considered a tumor marker for the diagnosis and prognosis of small cell lung cancer. Wang et al. also mentioned that NSE level was unrelated to EGFR mutation status.

Squamous cell carcinoma antigen is mainly present in the cytoplasm of squamous cell carcinoma, and high levels of SCCAg are often associated with poorly differentiated and advanced metastatic squamous cell carcinoma with high specificity.
proposed that in NSCLC patients, EGFR mutation was more common when SCCAg levels were below 1.5 ng/ml. A retrospective analysis\(^{21}\) also found that negative SCCAg result was an important predictor of EGFR mutation in patients with lung adenocarcinoma. However, Cho et al.\(^{32}\) suggested that serum SCCAg was not associated with EGFR mutation in NSCLC patients. Our study found that elevated SCCAg level was associated with a lower probability of EGFR mutation; moreover, the relationship persisted after adequate adjustment for confounding factors and was consistent across subgroups. The above results confirmed the potential value of SCCAg in predicting EGFR mutation in patients with lung adenocarcinoma and provided clinicians with a rapid, accurate, non-invasive and real-time monitoring method to predict EGFR mutation status, which facilitated the personalized diagnosis and treatment guidance.

There are still limitations of our study. First, this is a single-center retrospective study, and there might be bias in patient selection. Thus, the value of SCCAg for EGFR mutation prediction still needs to be confirmed by prospective studies. Second, the patient population of our study was Chinese patients with lung adenocarcinoma, which is not necessarily applicable to patients of other races and pathological types.

In conclusion, we discovered a negative association between SCCAg level and EGFR mutation probability in Chinese lung adenocarcinoma patients after fully adjusting for confounding factors: with the increase in SCCAg levels, the EGFR mutation probability gradually decreased. Our study fully explored the potential value of the serum tumor marker SCCAg in predicting EGFR mutation in lung adenocarcinoma, which can help improve the accuracy of clinical EGFR mutation prediction and guide the targeted therapy for lung adenocarcinoma patients.

**AUTHOR CONTRIBUTIONS**

LJJ and XNS contributed to the study concepts and the study design. SYZ, JXG, and RN contributed to data acquisition and reconstruction. JXG, RN, and JRY contributed to data analyses and interpretation. XNS contributed to the statistical analysis. JXG, RN, and JHM contributed to the manuscript preparation and editing, and review. All authors read and approved the final manuscript.

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**CONFLICT OF INTEREST**

The authors of this manuscript declare to have no conflict of interest related to this study.

**DATA AVAILABILITY STATEMENT**

The data supporting our findings are available upon request.

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**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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