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EGFR Mutation and FHIT Methylation: Inverse Relationship in Patients with Lung Adenocarcinoma and Tuberculosis

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Abstract: *Objective:* To investigate the genetic correlations between epithelial growth factor receptor (EGFR) mutation and FHIT methylation in patients diagnosed with lung adenocarcinoma (AC) and pulmonary tuberculosis (TB). *Methods:* The presence of EGFR mutations and the methylation status of the FHIT gene in patients presenting with AC and TB were analyzed. The correlation between TB status and the observed genetic and epigenetic variations was also examined. *Results:* Among the 90 patients included in the study, 38 exhibited EGFR mutations (14 among those with TB and 24 among those without TB), while 29 exhibited FHIT myelination (19 among those with TB and 10 among those without TB). Furthermore, the protein expression levels of EGFR and FHIT were significantly higher in patients diagnosed solely with AC compared to those presenting with both AC and TB. A robust inverse correlation was identified between TB status and the frequency of EGFR mutation ($P < 0.001$). Moreover, significant associations were observed between TB status and FHIT methylation ($P < 0.01$). *Conclusion:* The findings suggest a correlation between TB and the prevalence of EGFR mutation and FHIT methylation in the pathogenesis of AC.

Keywords: Lung cancer; Adenocarcinoma (AC); Tuberculosis (TB); Epithelial growth factor receptor (EGFR); Fragile histidine triad (FHIT)

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1. Introduction

In many countries, lung cancer remains the primary cause of cancer-related deaths. Despite advancements in detection and treatment, the five-year survival rate remains below 15%. China, particularly affected, exhibits a high incidence of malignant tumors, with lung cancer contributing to a significant portion, resulting in approximately 781,000 deaths annually. In Xinjiang, the incidence of lung cancer stands at 17.70 per 100,000 individuals, aligning with the national average [1,2]. Lung cancer is broadly categorized into two histological types: small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC), with adenocarcinoma (AC) being the predominant subtype within NSCLC, with its prevalence steadily increasing.

According to the World Health Organization, tuberculosis (TB) remains a significant global health concern, with 10.4 million reported cases in 2016, leading to 1.67 million deaths, including 600,000 cases of multidrug-resistant TB. In China, the incidence was 68 cases per 100,000, resulting in 38,000 fatalities, with a documented rate of multidrug-resistant TB cases at 6.6%. Xinjiang province exhibits the highest incidence nationwide, estimated at 181.42 per 100,000 individuals annually. Approximately 2%–8% of TB cases are complicated by lung cancer [3-5], with TB serving as a strong risk factor for its development. Recent molecular epidemiological studies have started to elucidate the precise gene mutations associated with the progression of cancer in pulmonary TB cases [6,7].

Furthermore, investigations into the connection between TB and DNA methylation have been underway, spurred by findings in animal studies linking DNA methylation and carcinogenesis [8-10]. Carcinomas commonly involve genetic and epigenetic alterations. Previous studies have indicated a prevalence of epithelial growth factor receptor (EFGR) mutations in AC patients [11], while genes associated with TB have shown methylation due to epigenetic modifications. However, data on distinct methylation patterns in AC based on TB status remain limited.

Lung AC harboring mutated EFGR has exhibited significant responses to tyrosine kinase inhibitors (TKIs), indicating a notable survival advantage. The efficacy of TKIs in tumor treatment largely hinges on the status of both EGFR mutation and gene amplification. This study aims to analyze lung AC with or without TB for EGFR mutations and the methylation status of the TB-specific FHIT tumor suppressor gene. The investigation seeks to elucidate the correlation between TB status and the association between genetic mutations and epigenetic alterations in lung AC.

2. Methods

The research comprised the examination of the study population and the subsequent DNA extraction process. A total of 90 lung specimens were acquired through surgical resection following histological diagnosis at the Eighth Affiliated Hospital of Xinjiang Medical University, with participants providing written informed consent. These samples were preserved at -80˚C until further analysis. All patients had not received any chemo- or radiotherapy before the surgery to prevent any changes in cell-cycle proteins due to DNA damage.

2.1. Pulmonary tuberculosis group (Group A)

Between 2020 and 2022, 30 pulmonary TB tissues were collected and confirmed pathologically. The patients, middle-aged or elderly individuals diagnosed with pulmonary TB, had undergone surgical resection or bronchoscopy at the Chest Hospital of Xinjiang Autonomous Region. Among the 30 patients (17 males and 13 females), aged between 45 and 75, with a mean age of 59.8, 4 were newly diagnosed cases, while 26 had received prior treatment. Acid-fast smear test results were positive for 8 cases, while the remaining 22 tested negative.

2.2. Adenocarcinoma group (Group B)

From 2020 to 2022, 30 lung cancer tissues from patients confirmed pathologically and treated with either surgical resection or bronchoscopy at Xinjiang Autonomous Region's Chest Hospital were obtained. The sample consisted of 18 males and 12 females, with an average age of 60.9 years (ranging from 45 to 80 years). Classification based on the 2015 World Health Organization (WHO) Lung Cancer Classification identified 9 stage I+II cases and 21 stage III+IV cases following the 8th edition of UICC's lung cancer staging guidelines (2017). Tumor diameters were ≤ 3 cm for 11 cases and ≥ 3 cm for the remaining 19 cases.

2.3. Tuberculosis plus lung adenocarcinoma group (Group C)

From 2020 to 2022, 30 tissues pathologically verified to have pulmonary TB and lung AC in patients who underwent surgical resection or bronchoscopy at the Chest Hospital of Xinjiang Autonomous Region were gathered. Inclusion criteria encompassed middle-aged or elderly patients meeting diagnostic criteria for both pulmonary TB and lung cancer, or those with a history of TB. The group, diverse in histological types, grades, and pathological stages, comprised 14 males and 16 females, with an average age of 60.5 years (range: 45–84 years). In 24 cases, TB and tumor were on the same side of the lungs, while 6 cases exhibited TB and tumor on opposite lungs. Exclusion criteria included recent myocardial infarction, unstable angina, severe organ complications or failures, Alzheimer's disease, medically proven mental disorders, and inability to undergo bronchoscopy or surgery. Patients with past or suspected HIV infections were also excluded.

2.4. Genomic DNA extraction

Genomic DNA extraction was conducted using a SepaGene kit. The analysis of mutations in exons 19, 20, and 21 of EGFR was conducted through PCR-single strand conformation polymorphism (SSCP) and direct sequencing analyses. Direct sequencing involved using small pieces of the gel containing the shift band detected by SSCP. Gel electrophoresis, data collection, and data analysis were performed using a Genetic Analyzer (PE Applied Biosystems, CA, USA) [12].

Methylation-specific PCR (MSP) determined the methylation status of FHIT after bisulfite treatment of DNA samples. The primer sequences are documented [13], and an unmethylated DAPK primer set verified bisulfite modification of all DNA samples.

2.5. Statistical analysis

Statistical analysis employed either the chi-squared test or Fisher's exact test with the SPSS 24.0 software. A *P*-value less than 0.05 indicated statistical significance.

3. Results

Of all the patients, 38 displayed EGFR mutations (14 patients with TB, and 24 patients without TB), accounting for 42% of cases, while 29 patients had FHIT myelination (19 patients with TB, and 10 patients without TB). Moreover, the protein expression of EGFR and FHIT was significantly higher in patients with AC as compared to the patients with AC and TB (**Figure 1**).

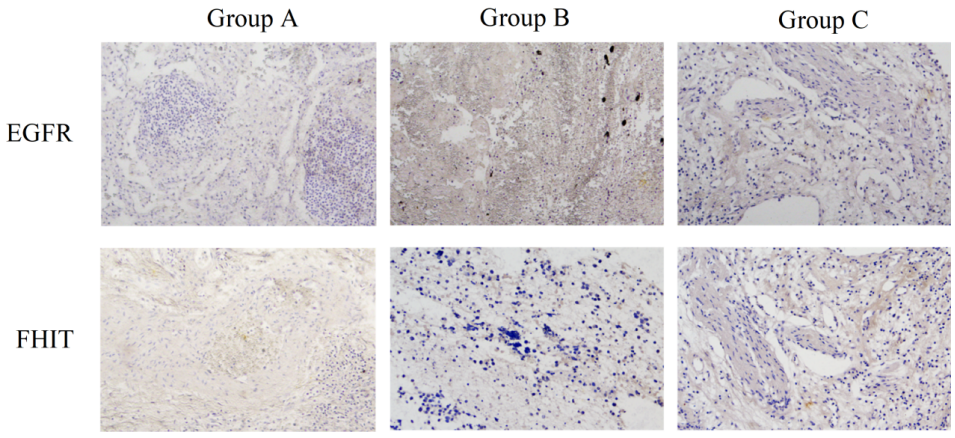

Figure 1. The protein expression of EGFR and FHIT in lung tissue was significantly higher in patients with AC compared to the patients with AC and TB

Among the 38 patients, 23 showed mutations of exon 19, 14 showed mutations of exon 20, and 18 showed mutations of exon 21. Frequencies of EGFR mutations were significantly higher on both exon 21 and exon 19 in patients with AC as compared to the patients with AC and TB, while there was no significant difference in the frequencies of EGFR mutations on exon 20 in patients with AC and patients with AC and TB (**Figure 2**).

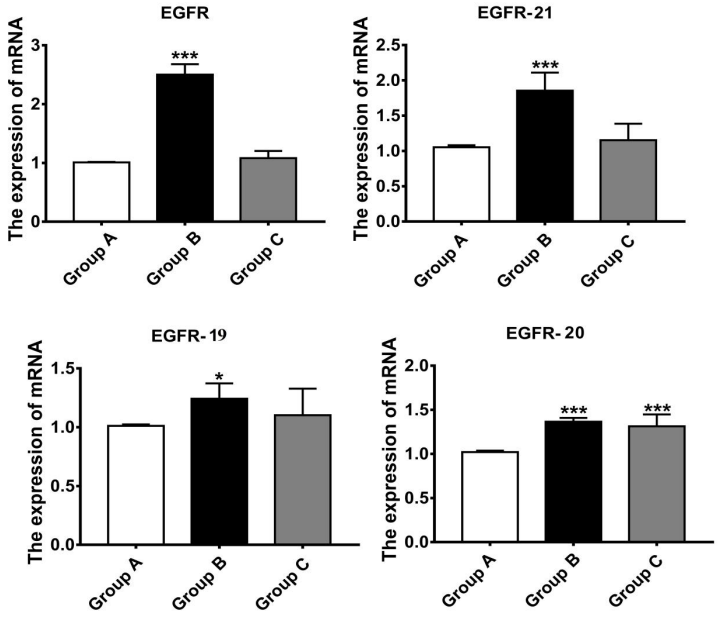

Figure 2. Frequencies of EGFR mutations were significantly higher on exon 19 and 21 in patients with AC compared to the patients with AC and TB

The FHIT gene exhibited methylation. A strong inverse correlation was found between tuberculosis status and frequency of EGFR mutation ($P < 0.001$), while significant correlations were observed between tuberculosis status and methylation of FHIT ($P < 0.01$). Furthermore, FHIT methylation frequency increased with tuberculosis status, while EGFR mutation frequency decreased with tuberculosis status (**Table 1** and **Figure 3**). A reverse correlation was observed between EGFR mutation and FHIT methylation.

Table 1. Status of EGFR mutation and FHIT myelination in patients with AC

		EGFR		*P* value
		Mutant	Wild type	
FHIT	Unmyelinated	32	26	< 0.01
	Myelinated	16	46	

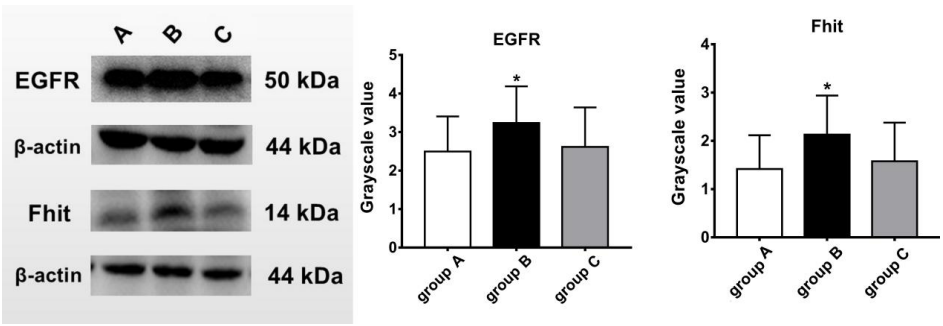

Figure 3. FHIT methylation frequency increased with TB status, while EGFR mutation frequency decreased with TB status

4. Discussion

Recent advances in our understanding of cell signaling pathways controlling cell survival have revealed genetic and regulatory aberrations that suppress cell death, promote cell division, and induce tumorigenesis. Among these discoveries is the EGFR, a transmembrane receptor tyrosine kinase protein expressed in various normal tissues, including epithelial, mesenchymal, and neurogenic tissue. Overexpression of EGFR has been reported in several human malignancies, including NSCLC. Lung AC harboring mutated EGFR has exhibited notable responses to TKIs, suggesting a significant survival advantage. The status of both EGFR mutation and gene amplification may be crucial in determining which tumors effectively respond to TKIs.

The observed pattern concerning TB-related mutations and methylations in this study indicates an increase in the frequency of alterations in FHIT methylation correlated with individual TB status and a tendency for the frequency of EGFR mutations to decrease inversely proportional to TB status. Unique methylation and mutation patterns of EGFR observed in TB and non-TB lung AC patients may be linked to their susceptibility to carcinogen exposure from TB. Based on these findings, our hypothesis suggests that TB may hinder the development of EGFR mutations.

Reports regarding the impact of lung AC and EGFR mutations on the incidence and outcomes of patients with a history of TB are limited. A study in the Taiwan region found a higher incidence of EGFR mutations in patients with lung AC exhibiting radiographic evidence consistent with previous tuberculosis pulmonary lesions (OR: 1.83 [0.92–3.62]) [14]. Another retrospective study analyzed the National Health Insurance Research Database of the Taiwan region and included 8,265 patients with NSCLC who received EGFR-TKIs between 1996 and 2000. This study reported a history of pulmonary TB associated with a poor clinical response to EGFR-TKIs in male patients but a better response in female patients [15].

FHIT methylation in TB patients showed a higher trend, while non-TB patients exhibited a lower trend in the development of EGFR mutation, as per the current study. Therefore, FHIT methylation could potentially suppress EGFR mutation. Studies have reported higher rates of methylation and mean methylation index in TB patients compared to non-TB patients, indicating a relationship between abnormal methylation and TB status [16-19]. Thus, the reduction in the overall methylation rate in TB suggests that methylation is a reversible epigenetic change that does not affect the DNA coding sequence [20]. From this perspective, the inhibition of EGFR mutation could be attributed to FHIT methylation, leading to an increase in EGFR mutations following TB.

Advancements in our understanding of cell signaling pathways have revealed genetic and regulatory abnormalities contributing to tumor formation, including EGFR, expressed in various normal epithelial, mesenchymal, and neurogenic tissues [21,22]. Overexpression of EGFR has been linked to the development of several human malignancies, including NSCLC [23]. Studies have shown that NSCLC patients with EGFR expression have lower survival rates, frequent lymph node metastasis, and poor response to chemotherapy. Lung AC harboring mutated EGFR responds well to TKIs [24-26], but there is a significant survival advantage for non-smoking Asian women with AC, particularly those with bronchioloalveolar carcinoma [27]. Two oral anti-cancer drugs, gefitinib and erlotinib, have been approved for advanced NSCLC treatment, with mutations in EGFR found in some lung cancers [28]. Efforts have been made to identify clinical, morphological, and molecular factors predicting response rates to these drugs. In NSCLC cases, overexpression of EGFR or mutations in intracellular EGFR have been observed in 43%–89% of cases. Other studies report that a quarter of NSCLC cases have mutations in the EGFR tyrosine kinase domain, and these mutations are associated with increased receptor expression in 75% of cases [29]. The majority of known EGFR tyrosine kinase domain mutations involve short deletions in exon 19 or point mutations in exon 21 [30]. Results in our study indicate a significantly higher frequency of EGFR mutation in patients with lung AC secondary to TB, suggesting a poor

response to EGFR-TKIs, resulting in lower survival rates, frequent lymph node metastasis, and poor response to chemotherapy.

However, the study was limited by a small sample size, and approximately 25% of cases showed no evidence of EGFR mutation or FHIT methylation. Further research is needed to confirm these findings. In summary, two distinct pathways are involved in the development of AC in TB patients, namely EGFR mutation and FHIT methylation. The pathogenesis of AC is associated with the frequencies of EGFR mutation and FHIT methylation, which are also correlated with lung TB and inversely correlated with each other.

Acknowledgments

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Chest Hospital of Xinjiang Uygur Autonomous Region (Urumqi, China). The methods used in this study were performed in accordance with relevant guidelines and regulations. Written consent was obtained from the participants.

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Disclosure statement

The authors declare no conflict of interest.

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