

## RESEARCH ARTICLE

# Comparison of Epidermal Growth Factor Receptor Mutations between Primary Tumors and Lymph Nodes in Non-small Cell Lung Cancer: a Review and Meta-analysis of Published Data

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### Abstract

**Background:** Epidermal growth factor receptor (EGFR) mutations in non-small cell lung cancer (NSCLC) can predict the clinical response to tyrosine kinase inhibitor (TKI) therapy. However, EGFR mutations may be different in primary tumors (PT) and metastatic lymph nodes (MLN). The aim of this study was to compare EGFR mutations between PT and the corresponding MLN in NSCLC patients, and provide some guidelines for clinical treatment using TKI therapy. **Materials and Methods:** A systematic review and meta-analysis was performed with several research databases. Relative risk (RR) with the 95% confidence interval (CI) were used to investigate the EGFR mutation status between PT and the corresponding MLN. A random-effects model was used. **Results:** 9 publications involving 707 patients were included in the analysis. It was found that activation of EGFR mutations identified in PT and the corresponding MLN was 26.4% (187/707) and 19.9% (141/707), respectively. The overall discordance rate in our meta-analysis was 12.2% (86/707). The relative risk (RR) for EGFR mutation in PT relative to MLN was 1.33 (95% CI: 1.10-1.60; random-effects model). There was no significant heterogeneity between the studies ( $I^2=5%$ ,  $p=0.003$ ). **Conclusions:** There exists a considerable degree of EGFR mutation discrepancy in NSCLC between PT and corresponding MLN, suggesting that tumor heterogeneity might arise at the molecular level during the process of metastasis.

**Keywords:** Non-small cell lung cancer - EGFR - mutation - lymph node - metastasis

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### Introduction

Lung cancer is the leading cause of cancer-related mortality throughout the world and is expected to remain a major health problem for the foreseeable future (Jemal et al., 2009). Non-small cell lung cancer (NSCLC) accounts for 75-80% of all lung cancer cases (Hansen, 2002). Patients with early stage NSCLC have long-term survival with surgical resection. However, most NSCLC patients were at an advanced stage at the time of diagnosis, usually with a poor prognosis (Wang et al., 2010). For stage III/IV NSCLC, platinum-based combined chemotherapy has been considered the standard therapeutic modality (Felip et al., 2005). However, the efficacy of such treatment strategies has reached a plateau (Molina et al., 2008). The discovery of epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), such as gefitinib or erlotinib, is a milestone in the development of NSCLC treatment, especially in a genetically defined subset of patients who are females, nonsmokers, Asians, and have adenocarcinoma (Mok et al., 2009). Several clinical trials have shown that most patients with EGFR-activating mutations responded well to EGFR-TKIs (Morita et al.,

2009; Chung et al., 2010; Chu et al., 2013). The objective response rate (ORR) of gefitinib was 71.2%. However, the ORR of gefitinib for NSCLC patients with wild type EGFR was less than 10% (Mok et al., 2009). So it is necessary to identify the mutation status of EGFR for selection of patients who are more likely to benefit from TKIs.

Generally, the EGFR mutation status is determined based on the analysis of primary tumors. However, tissue specimens are not easily obtained for advanced NSCLC patients with unresectable lung cancer. Non-surgical materials such as biopsy specimens, lymph nodes, pleural effusions, and sera may be used for genetic analysis as reported in prior studies (Sasaki et al., 2003; Shih et al., 2006; Hung et al., 2006). Despite a large number of studies performed in NSCLC patients, whether or not the EGFR mutation status is concordant between primary tumors and metastatic specimens remains controversial (Matsumoto et al., 2006; Daniele et al., 2009; Cortot et al., 2010). To synthesize the clinical trial evidence available, we performed a meta-analysis of 9 studies to clarify the prevalence of the EGFR mutation status in NSCLC patients matched for primary tumors (PT) and metastatic lymph nodes (MLN).

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**Materials and Methods**

*Search strategies and selection criteria*

To find relevant articles, we performed an Internet search of Medline (using PubMed as the search engine), the Cochrane library, the Embase database, the American Society of Clinical Oncology (ASCO, www.asco.org), and the European Society for Medical Oncology (ESMO, www.esmo.org). The keywords and text words used for searching were non-small cell lung cancer or NSCLC, epidermal growth factor receptor or EGFR, and lymph node. Deadline for trial publication and/or presentation was November 2013. The language was limited to English.

Two authors independently collected and extracted data carefully according to the following criteria: original study; patients with pathologically confirmed NSCLC; samples for mutation analysis obtained from biopsy or surgical tumor tissue specimens and paired lymph node specimens; and sufficient data on the mutational status of EGFR exons 18-21 between primary tumors and lymph nodes for estimating relative risk (RR) and its corresponding 95% confidence interval (CI).

*Data extraction and quality assessment*

Data of each trial, including author's name, year of publication, pathological type, different analysis data of EGFR mutation between primary tumors and matched lymph nodes, were extracted by two investigators independently. Most trials analyzed exons 18 and 21 for EGFR mutations, and some trials also included exons 18 and 20. Based on the findings obtained by the molecular analysis methods such as direct sequencing, high-resolution melting method, quantitative real-time PCR (qPCR), peptide nucleic acid-locked nucleic acid

polymerase chain reaction (PNA-LNA PCR) and TaqMan real-time PCR, EGFR mutation was defined as positive in the presence of a mutation, or as negative in the absence of mutation. The difference in EGFR mutation detection between PT and corresponding MLN was classified as mutation inconsistent. In this analysis, EGFR gene amplification, protein expression and the number of gene copies were not topics of interest. A total of 9 studies were included in this study. Table 1 illustrates the main characteristics of the patients included in the analysis. The mean NOS score for these studies was 6.3 (scores <7=low quality; >7=high quality, and maximum score=8).

*Statistical analysis*

A meta-analysis of RR was performed, and the associated 95% CIs was used to assess the mutation status of EGFR between PT and matched MLN. Statistical analysis was performed using the RevMan 5 software. The pooled RR for the risk was calculated using a Mantel-Haenszel method and a random-effects model. P values for all comparisons were two-tailed and statistical significance was defined as a P<0.05. Begg tests were performed to examine whether or not there was a publication bias.

**Results**

*Search results and description of the studies.*

A total of 219 potentially relevant published articles were retrieved electronically (Figure 1). Of these, 177 articles were removed by screening the title and abstract. The remaining 42 articles were selected for analysis and evaluated in greater detail by reviewing the full articles. After the selection procedure, 33 articles were excluded for the following reasons: 5 articles were excluded because they lacked paired comparison of the mutation status between PT and MLN specimens, and 4 other articles were excluded because we were not interested in gene expression or amplification in this meta-analysis. In addition, 22 non-relevant citations were excluded by careful review of the full articles. Finally, the remaining 9 trials including 707 patients that met the inclusion criteria were included in our meta-analysis. The characteristics of the eligible studies are shown in Table 1.

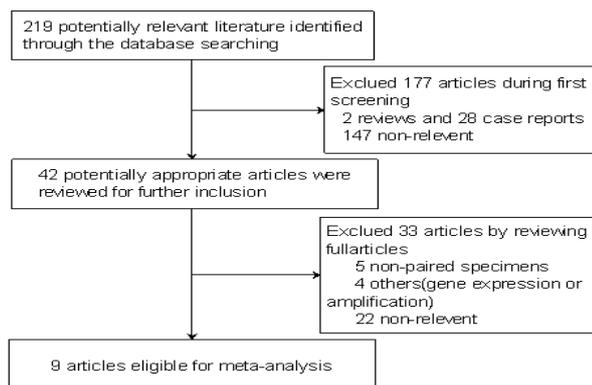
*Analysis of the 9 studies*

EGFR mutation in the 9 trials was examined on the basis of exons 18-21. Activation of EGFR mutation identified in PT and MLN was 26.4% (187/707) and 19.9%

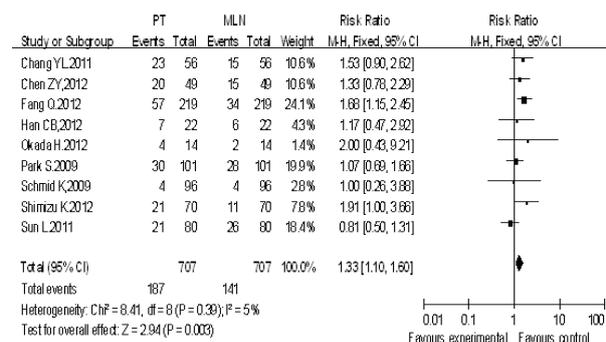
**Table 1. Overview of Studies in the Pooled Analysis**

| Study/year      | Histology | Pairs | LN<br>n (%) | LN<br>n (%) | Inconsistency<br>n (%) |
|-----------------|-----------|-------|-------------|-------------|------------------------|
| Chang YL, 2011  | NSCLC     | 56    | 23 (41.1)   | 15 (26.8)   | 16 (28.6)              |
| Chen ZY, 2012   | AD        | 49    | 20 (40.8)   | 15 (30.6)   | 5 (10.2)               |
| Fang Q, 2012    | NSCLC     | 219   | 57 (26.0)   | 34 (15.5)   | 23 (10.5)              |
| Han CB, 2012    | NSCLC     | 22    | 7 (31.8)    | 6 (27.3)    | 1 (4.5)                |
| Okada H, 2012   | NSCLC     | 14    | 4 (28.6)    | 2 (14.3)    | 1 (7.1)                |
| Park S, 2009    | NSCLC     | 101   | 30 (29.7)   | 28 (27.7)   | 17 (16.8)              |
| Schmid K, 2009  | AD        | 96    | 4 (4.2)     | 4 (4.2)     | 6 (6.3)                |
| Shimizu K, 2012 | NSCLC     | 70    | 21 (30.0)   | 11 (15.7)   | 10 (14.3)              |
| Sun L, 2011     | NSCLC     | 80    | 21 (26.3)   | 26 (32.5)   | 7 (8.6)                |

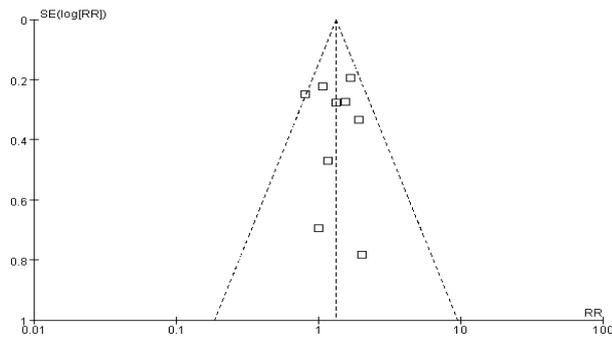
AD, Adenocarcinomas; PT, Primary tumor; LN, Lymph node



**Figure 1. Flow Chart of Trial Selection Process**



**Figure 2. EGFR Mutation between PT and Corresponding MLN**



**Figure 3. Funnel Plot of Publication Bias in the Meta-analysis**

(141/707), respectively. The overall discordance rate in our meta-analysis was 12.2% (86/707) (Table 1). The RR of EGFR mutation in PT relative to matched MLN was 1.33 (95% CI: 1.10-1.60; random-effects model) (Figure 2). There was no significant heterogeneity between the studies ( $I^2 = 5\%$ ,  $p = 0.003$ ).

#### Bias analysis

To reduce publication bias, we conducted a more detailed literature search. Begg's funnel plot was performed to assess the publication bias of the literature in this meta-analysis (Figure 3). The shape of the funnel plot did not reveal any evidence of obvious asymmetry. No publication bias analysis for RR was found according to Begg test ( $p = 0.003$ ).

#### Discussion

This meta-analysis provides a cumulative estimate of somatic mutations of EGFR in NSCLC between PT and the corresponding MLN. Obviously, evidence in the findings of molecular analysis was increased by the incorporation of the results from the 9 trials in 707 patients. We confirmed an overall discordance rate of 12.2% in the 707 patients. The results suggest that heterogeneity of EGFR gene mutation exists in PT and corresponding MLN.

Recently, studies on intratumoral and intertumoral heterogeneity have increased gradually (Kidd et al., 2008; Nakano et al., 2008; Wang et al., 2013). For clinical analysis, TKIs resistance in a proportion of patients with EGFR mutation is always explained by the heterogeneity of EGFR mutation between PT and metastatic lesions (Park et al., 2009), or even between parts of PT (Sakurada et al., 2008). Several reasons for discordance of molecular biomarkers associated with EGFR mutation between PT and LNM may be attributable to the presence of intratumoral heterogeneity of EGFR mutations or to technical limitations of the methods used for the assessment of EGFR mutations or to changes in EGFR mutations during disease progression and metastasis. As for intratumoral heterogeneity, Schmid (Schmid et al., 2009) also reported a discordance rate of 6.3% in 96 white patients. However, some studies have shown that the heterogeneous distribution of EGFR mutations is extremely rare (Yatabe et al., 2011). A combination of EGFR-mutated and wild-type cells was

detected with DHPLC and ARMS by Hua (Bai et al., 2013), who reported that approximately 30% Chinese patients with advanced NSCLC presented intratumoral EGFR mutational heterogeneity, indicating a difference in the frequency of EGFR mutation between the two ethnic groups. Of course, a more sensitive method for detecting EGFR mutations is needed.

EGFR gene detection methods are constantly explored by researchers. For molecular applications, isolation of DNA is challenging, because the nucleic acid is degraded into small fragments in part of samples. Cobas method could yield more DNA from biopsy specimens, and gain much better EGFR mutation results (Hu et al., 2014). Currently, direct sequencing for detection of EGFR mutation has been recognized as the gold standard in the prediction of TKI treatment responses (Sun et al., 2011; Chang et al., 2011; Han et al., 2012). However, direct sequencing is expensive, time-consuming and associated with a higher frequency of false-negative results. More importantly, the sensitivity of direct sequencing is suboptimal for clinical tumor samples. It is recommended that mutant DNA should comprise more than 25% of the total DNA for the convenience of detection (Pao et al., 2007). This means that the specimen should contain a mixture of tumor and normal cells, because mutation detection by direct sequencing may lead to "false-negative" results. Previous studies reported that the mutant-enriched PCR method (Asano et al., 2006), ARMS method (Ho et al., 2013), high-resolution melting method (Chen et al., 2012; Jing et al., 2013), PNA-LNA clamp method (Nagai et al., 2005; Hironobu et al., 2012; Katsuhiko et al., 2013), TaqMan real-time PCR (Qin et al., 2012; Didelot et al., 2012), and Scorpion amplification refractory mutation system method (Gao et al., 2012) are more sensitive than the direct sequencing method. Indeed, some limitations of direct sequencing should be acknowledged. First, a proportion of unknown mutations cannot be detected. Second, most methods of mutation analysis rely on mutation-specific primers and probe to detect small variable insertions or deletions. We speculate that the discordance detection rate of EGFR mutations between PT and MLN in NSCLC with the combination of direct sequencing and other sensitive methods would be higher than that of direct sequencing or other sensitive methods alone.

As for the occurrence of changes in EGFR mutations during the disease progression and metastasis, Chen (Chen et al., 2012) demonstrated the discordance of EGFR present in primary lesions at different times. In addition, they observed that the discordance rate of patients without exposure to any systemic therapy and those with exposure was 9.3% (4/43) and 13.6% (8/59), respectively. The highest discordance rate 26.3% (10/38) was observed in patients exposed to TKIs. This result may help explain why some mutation-negative patients were responsive to TKIs whereas some mutation-positive patients were resistant. It is recommended that tumors that progress during TKI treatment should be re-biopsied (Sequist et al., 2011). In clinical practice, mediastinal lymph nodes were found to be involved in 28-38% NSCLC patients at the time of diagnosis (Silvestri et al., 2007). Previous reports

indicate that EBUS-TBNA assessment of EGFR mutation is useful for patients with unresectable lung cancer in whom tissue specimens cannot be easily obtained (Neal et al., 2012; Esterbrook et al., 2013). However, we found that tumors metastasized to lymph nodes did not always show the same gene status as their primary compartments. Also, the response rate to EGFR-TKI tended to be higher in patients with EGFR mutations in MLN (Chen et al., 2012). This means that the EGFR mutation status in MLN may be a better predictive marker of the response to EGFR-TKI therapy in NSCLC patients.

Several limitations should be taken into account in relation to this meta-analysis. Firstly, the potential source of heterogeneity is a potential problem, including differences in study design, age distribution, smoking history, pathological type of lung cancer, and stage of lung cancer. Secondly, the included studies lacked homogeneity concerning the methods for EGFR mutation analysis of paired specimens. In addition, inconsistent mutation cannot be reconfirmed by other methods, which may result in potential under-estimation and/or overestimation of the true incidence of EGFR mutation. Thirdly, we were unable to conduct a subgroup analysis due to the lack of detailed description about MLN, including the metastatic site and the degree of differentiation. Finally, a possible publication bias might have been introduced because we included only English-language publications, though an examination of funnel plots showed no evidence of a publication bias.

In conclusion, this meta-analysis indicates that discordance of EGFR mutations between PT and corresponding MLN in NSCLC patients. Determination of the EGFR mutation status in both primary and metastatic tumors of NSCLC patients with MLN may be critical for making meaningful decisions regarding the appropriate use of targeted therapies.

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