Association of EGFR Mutation or ALK Rearrangement With Expression of DNA Repair and Synthesis Genes in Never-Smoker Women With Pulmonary Adenocarcinoma

Shengxiang Ren, MD1; Xiaoxia Chen, MD1; Peng Kuang, MD1; Limou Zheng, PhD2; Chunxia Su, MD1; Jiayu Li, MD1; Bing Li, MD1; Yongshen Wang, MD1; Lu Liu, MD1; Qiong Hu, MD1; Jie Zhang1; Liang Tang1; Xuefei Li, PhD3; Caicun Zhou, MD, PhD1; and Gerald Schmid-Bindert, MD4

BACKGROUND: Epidermal growth factor receptor (EGFR) mutation and anaplastic lymphoma kinase (ALK) rearrangement may predict the outcome of targeted drug therapy and also are associated with the efficacy of chemotherapy in patients with nonsmall cell lung cancer (NSCLC). The authors of this report investigated the relation of EGFR mutation or ALK rearrangement status and the expression of DNA repair or synthesis genes, including excision repair cross-complementing 1 (ERCC1), ribonucleotide reductase subunit M1 (RRM1), thymidylate synthetase (TS), and breast cancer-early onset (BRCA1), as a potential explanation for these observations. METHODS: In total, 104 resected lung adenocarcinomas from women who were nonsmokers were analyzed concurrently for EGFR mutations, ALK rearrangements, and mRNA expression of the ERCC1, RRM1, TS, and BRCA1 genes. EGFR mutations were detected with a proprietary detection kit. ALK rearrangements were detected by polymerase chain reaction analysis, and genetic mRNA expression was detected by real-time polymerase chain reaction analysis. RESULTS: Of 104 patients, 73 (70.2%) had EGFR mutations, and 10 (9.6%) had ALK rearrangements. ERCC1 mRNA levels in patients who had EGFR mutations were 3.44 ± 1.94 × 10−3, which were significantly lower than the levels in patients who were positive for ALK rearrangements and in patients who were negative for both biomarkers (4.60 ± 1.95 × 10−3 and 4.95 ± 2.33 × 10−3, respectively; \( P = .010 \)). However, TS mRNA levels were significantly lower in patients who had EGFR mutations (1.15 ± 1.38 × 10−3 vs 2.69 ± 3.97 × 10−3; \( P = .006 \)) or ALK rearrangements (1.21 ± 0.78 × 10−3 vs 2.69 ± 3.97 × 10−3; \( P = .020 \)) than in patients who were negative for both biomarkers. CONCLUSIONS: NSCLC specimens that harbored activating EGFR mutations were more likely to express low ERCC1 and TS mRNA levels, whereas patients with NSCLC who had ALK rearrangement were more likely to express low TS mRNA levels.

INTRODUCTION
Despite ongoing efforts to reduce smoking, lung cancer is the leading cause of cancer-related deaths, with 1.4 million deaths worldwide annually.1 Nonsmall cell lung cancer (NSCLC) accounts for approximately 80% of primary lung cancer cases, and approximately 66% of patients with NSCLC are diagnosed at an advanced stage.1 Although several active anticancer agents are available, platinum-based chemotherapy, including third-generation agents like taxanes, gemcitabine, vinorelbine, and pemetrexed, is still the standard first-line therapy for patients with metastatic or recurrent disease and produces improved overall survival and life quality.2-4 However, the outcome of patients with advanced NSCLC remains very poor (median survival, 8-12 months).2,5 Thus, more efficacious and less toxic strategies for patients with NSCLC are urgently needed.

With the recent advances in pharmacogenomics research, it is possible to tailor chemotherapy for patients with advanced NSCLC to improve efficacy and reduce toxicity according to the expression levels of 1 or several genes.5-8 With the objective of improving the efficacy of platinum-based chemotherapy, a phase 3 trial was conducted based on chemotherapy tailored to excision repair cross-complementation group 1 (ERCC1) mRNA expression levels in patients with advanced NSCLC.5 Patients in the control arm received docetaxel plus cisplatin, patients with low ERCC1 levels received docetaxel and cisplatin, and patients with high levels of ERCC1 received gemcitabine and docetaxel. The results indicated

DOI: 10.1002/cncr.27603, Received: January 24, 2012; Revised: March 26, 2012; Accepted: March 27, 2012. Published online May 8, 2012 in Wiley Online Library (wileyonlinelibrary.com)
Likewise, together with other authors, we9,10 previously has promise for producing improved patients outcome. 

Levels in patients with advanced NSCLC is feasible and survival.12,15 Echinoderm microtubule associated protein the DNA synthesis and DNA damage repair pathways.7,8

Along with the DNA repair genes, it has been reported that epidermal growth factor receptor (EGFR) mutations can predict the efficacy of EGFR-tyrosine kinase inhibitors (TKIs), and such mutations were enriched in Asian women with pulmonary adenocarcinoma who were never-smokers. Recently, several phase 3 studies11-15 compared first-line EGFR-TKIs, such as gefitinib and erlotinib, with doublet platinum-based chemotherapy in the treatment of patients with advanced NSCLC who harbored activating EGFR mutations, and the results indicated that EGFR-TKIs produced significantly higher response rates and longer progression-free survival (PFS) than traditional chemotherapy in this selected population. Consequently, 2011 National Comprehensive Cancer Network guidelines16 recommend EGFR-TKIs as the first-line choice for patients with advanced NSCLC who have activating EGFR mutations. Also in patients with EGFR mutations, several studies have indicated that chemotherapy may result in higher response rates,11 longer PFS,15,17 or longer overall survival.12,15 Echinoderm microtubule associated protein line 4-anaplastic lymphoma kinase (EML4-ALK) fusion is another important finding in the development of lung cancer that can inhibit apoptosis and the promotion of cellular proliferation through the activation of the downstream phosphoinositide-3-kinase/v-akt murine thymoma viral oncogene homolog 1 (PI3K/Akt) and microtubule-associated protein kinase (MAPK) signaling pathways.18 Similar to EGFR mutations, previous results from limited samples indicated that the frequency of EML4-ALK fusion was increased in individuals with adenocarcinomas, in young adult patients, and in individuals who never smoked or who were light smokers.18 Recently, based on the excellent efficacy of crizotinib, an ALK inhibitor, in the treatment of patients with advanced NSCLC who had EML4-ALK fusion,19,20 the US Food and Drug Administration has approved its use in clinical practice. It is noteworthy that several studies have reported that patients who harbored ALK translocations also gained more benefit from treatment with pemetrexed.21,22

The clinical implications of these findings remain unclear. We hypothesized an association between pharmacogenomics, such as DNA repair capacity and synthesis, and EGFR mutation status or ALK rearrangement status as a possible explanation. The objective of this study was to investigate the association of mRNA expression of the ERCC1, RRM1, thymidylate synthetase (TS), and breast cancer-early onset (BRCA1) genes with EGFR mutation or ALK rearrangement status in 104 East Asian women nonsmokers who had resected pulmonary adenocarcinomas from a single institution (Tongji University, Shanghai, China).

**MATERIALS AND METHODS**

**Specimen Collection**

Eligible patients were women with NSCLC who underwent surgery and had a histopathologic diagnosis of adenocarcinoma. Tumor tissues were collected within half an hour of resection and were fixed in 10% neutral-buffered formalin and then stored as paraffin-embedded archival until use. All tissues were reviewed by pathologists for confirmation of tumor histology and tumor content. Paraffin sections were then cut at 10 μm thickness, and DNA and RNA were isolated from 10 slides of the paraffin-embedded specimens. Patients were considered never-smokers in this study if they reported smoking <100 cigarettes in their lifetime. Histology was based on criteria of the World Health Organization, and the TNM classification was determined according to version 7 the International Association for the Study of Lung Cancer staging system. This study was approved by the Ethics Committee of Shanghai Pulmonary Hospital, Tongji University (Shanghai, China); and written informed consent was obtained from each participant before the initiation of any study-related procedure.

**Mutational Analyses**

All mutational analyses were performed in Tongji University Medical School Cancer Institute (Shanghai, China). Genomic DNA or RNA was extracted from lung tumors using standard protocols (RNeasy Mini Kit and QiAamp DNA Mini Kit; Qiagen, Hilden, Germany). EGFR (exons 18-22) was sequenced using genomic DNA. Cycle sequencing of the purified polymerase chain reaction (PCR) products was carried out with PCR primers using the commercially available ADx Mutation Detection Kits (Amoy Diagnostics Company Ltd., Xiamen, China). The
Ct values that we used to determine whether a sample was positive or negative were based on extensive validation.

**Detection of ALK Rearrangements in Clinical Specimens**

ALK rearrangements were detected readily by PCR with the ADx EML4-ALK Fusion Gene Diagnostic Kit (Amoy Diagnostics Company Ltd.). Briefly, total RNA was extracted with the Qiagen RNeasy FFPE Kit (catalog no. 73504), and mRNA was transcribed to cDNA at 42°C for 1 hour. β-Actin was used as the internal control. The PCR conditions were as follows: an initial denaturation step at 95°C for 5 minutes was followed 95°C for 25 seconds, 64°C for 20 seconds, and 72°C for 20 seconds to ensure the specificity; and 31 cycles at 93°C for 25 seconds, 60°C for 35 seconds, and 72°C for 20 seconds for data collection and to determine sensitivity. Qualitative judgments were based on the mutation fluorescence signal.

**Real-Time Polymerase Chain Reaction Analysis for ERCC1, RRM1, TS, and BRCA1 mRNA Expression**

Relative cDNA quantification for the *ERCC1*, *RRM1*, *TS*, and *BRCA1* genes was done using a fluorescent, real-time detection method (LightCycler 2.0; Roche Applied Science, Penzberg, Germany) using an internal reference gene (β-actin) as a control. The details of this process were published previously in studies from our institute. Amplification was carried out in a total volume of 25 μL containing 0.25 μM of each primer, 0.02 mM deoxyribonucleotide triphosphates, 1 mM MgCl₂, 1.25 U Taq polymerase, and 5 × PCR buffer. The PCR program was initiated with a 1-minute denaturation step at 95°C. The DNA was amplified by 1 cycle at 95°C for 5 seconds and 50 cycles at 92°C for 40 seconds, followed by elongation of at 60°C for 40 seconds. All gene expression analyses were performed in a blinded fashion in which the laboratory investigators were unaware of the clinical data.

**Statistical Analysis**

For statistical analyses, we used the SPSS statistical software package (version 13.0; SPSS, Inc., Chicago, Ill). Chi-square tests or Fisher exact tests were used to analyze correlations between EGFR mutation or ALK rearrangement status and clinicopathologic variables. The Mann-Whitney *U* test was used to determine significant associations between continuous variables (ie, gene expression) and dichotomous variables (ie, mutation status). All statistical tests were conducted at a 2-sided level of significance of *P* < .05.

---

**RESULTS**

**Assembly of Tumor Samples**

From July 2008 to May 2010, 135 resected specimens from women with lung adenocarcinoma were collected consecutively at the Shanghai Pulmonary Hospital, Tongji University, Shanghai, China. All patients (100%) were ethnic Chinese (Han). Of these, 104 patients were included in this study based on the following criteria: a rereview confirmed a pathologic diagnosis of lung adenocarcinoma, the tumor specimen contained a minimum of 30% tumor cells, enough tissue was available for comprehensive analysis, the patient was a never-smoker, and the patient had not received any neoadjuvant treatment or anticancer drugs. Detailed clinical characteristics are listed in Table 1.

**EGFR Mutation and ALK Rearrangement Status**

Seventy-three of 104 tumors (70.2%; 95% confidence interval, 61.4%-79%) harbored EGFR kinase domain mutations. Among these, 28 mutations were deletions in exon 19, 44 were leucine to arginine codon 858 (L858R) missense changes, 8 were threonine to methionine codon 790 (T790M) mutations, and 7 tumors contained 2 different mutations, such as an exon 19 deletion together with T790M mutation or an L858R with T790M mutation. The EGFR mutation status had no significant association with clinical characteristics in terms of age, performance status, disease stage, or tumor differentiation (see Table 2).

ALK rearrangement was observed in 9.6% of tumors (10 of 104; 95% confidence interval, 3.95%-15.28%).

---

**Table 1. Clinical Characteristics of Women Never-Smokers With Lung Adenocarcinoma (N = 104)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>104</td>
</tr>
<tr>
<td>Age: Median (range), y</td>
<td>59.5 (27-78)</td>
</tr>
<tr>
<td>ECOG performance status</td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>89 (85.6)</td>
</tr>
<tr>
<td>2</td>
<td>15 (14.4)</td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>51 (49)</td>
</tr>
<tr>
<td>II</td>
<td>14 (13.5)</td>
</tr>
<tr>
<td>III</td>
<td>24 (23.1)</td>
</tr>
<tr>
<td>IV</td>
<td>15 (14.4)</td>
</tr>
<tr>
<td>Tumor differentiation</td>
<td></td>
</tr>
<tr>
<td>Well differentiated</td>
<td>39 (37.5)</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>34 (32.7)</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>31 (29.8)</td>
</tr>
</tbody>
</table>

Abbreviations: ECOG, Eastern Cooperative Oncology Group.
The median age was 54 years in patients with ALK rearrangement and <65.1 years in patients without ALK rearrangement. ALK rearrangement had no statistical correlation with other clinical characteristics, such as performance status, disease stage, and tumor differentiation (see Table 2). EGFR mutation and ALK rearrangement were mutually exclusive among the 104 patients.

**The Relation of mRNA Expression Levels of ERCC1, RRM1, TS, and BRCA1 and EGFR Mutation or ALK Rearrangement Status**

Expression levels of ERCC1, RRM1, TS, and BRCA1 mRNA are listed in Table 3. By using EGFR mutation status and ALK rearrangement status to divide the patients into groups, we identified 3 groups: an EGFR mutation group, an ALK-positive group, and a group that was negative for both biomarkers. The ERCC1 mRNA level in patients with EGFR mutations was \(3.44 \pm 1.94 \times 10^{-3}\), which was significantly lower than the level in patients who ALK-positive and negative for both biomarkers (4.60 \(\pm 1.95 \times 10^{-3}\) and 4.95 \(\pm 2.33 \times 10^{-3}\), respectively; \(P = .010\), see Figure 1). However, levels of TS mRNA were significantly lower in patients who were positive for EGFR mutations (1.15 \(\pm 1.38 \times 10^{-3}\) vs 2.69 \(\pm 3.97 \times 10^{-3}\); \(P = .006\), see Figure 2) or for ALK rearrangements (1.21 \(\pm 0.78 \times 10^{-3}\) vs 2.69 \(\pm 3.97 \times 10^{-3}\); \(P = .020\), see Figure 2) than in the patients who were negative for both biomarkers. However, RRM1 (10.48 \(\pm 5.80 \times 10^{-3}\) vs 13.27 \(\pm 11.28 \times 10^{-3}\) vs 13.27 \(\pm 11.28 \times 10^{-3}\); \(P = .808\) and BRCA1 (0.57 \(\pm 2.39\) vs 0.20 \(\pm 0.60\) vs 1.12 \(\pm 5.13\); \(P = .828\) mRNA expression levels were not correlated significantly with different EGFR mutation or ALK rearrangement status, although they were lower numerically in patients who had EGFR mutations than in the group that was negative for both biomarkers.

**DISCUSSION**

In this article, we report for the first time an association between EGFR mutation and ALK rearrangement status and expression levels of mRNA in the ERCC1, RRM1, TS and BRCA1 genes in 104 East Asian nonsmoking women who had resected pulmonary adenocarcinomas. In this preselected population, 73 tumors (70.2%) were positive for EGFR mutations, and 10 tumors (9.6%) were positive for ALK rearrangements. We observed that NSCLC specimens with EGFR mutations were more likely to express low ERCC1 and TS mRNA levels, whereas specimens that were positive for ALK rearrangements were more likely to express low levels of TS mRNA.

Pulmonary adenocarcinoma among Asian women who were nonsmokers was regarded first as a separate entity in the Iressa Survival Evaluation in Lung Cancer
In that preselected population, EGFR mutations (ie, deletions in exon 19 and L858R point mutations in exon 21) were enriched and were highly associated with increased sensitivity to EGFR-TKIs. More recently, previous results from limited samples indicated that the frequency of EML4-ALK fusion was increased in patients with adenocarcinoma, in young adult patients, and in individuals who had never smoked or who were light smokers. Sun et al observed that the EGFR mutation rate was as high as 82.9% in pulmonary adenocarcinoma samples from 41 Chinese women who were nonsmokers. Furthermore, Wu et al reported that, in patients with wild-type EGFR, the ALK rearrangement rate was 34% in a Taiwanese population. Consistent with these results, our findings suggest that the majority of lung adenocarcinomas from nonsmoking East Asian women can be defined molecularly by targetable oncogenic mutant kinases, mainly EGFR mutation and ALK rearrangement.

With the successful introduction of personalized, molecular-targeted agents like EGFR-TKIs and ALK inhibitors into clinical practice for the treatment of advanced NSCLC, more and more patients are identified who harbor EGFR mutations or ALK rearrangements before the initiation of therapy. It is noteworthy that these biomarkers reportedly not only were able to predict the efficacy of the targeted drugs but also appeared to be associated with the outcome of chemotherapy. In the Iressa Pan-Asia Study, efficacy in the chemotherapy arm was surprisingly good for the EGFR mutation subgroup; the overall response rate to chemotherapy in patients who had tumors with EGFR mutations was 47.3% compared with 23.5% in patients who had wild-type EGFR. Moreover, Wu et al reported that EGFR mutation-positive patients who received pemetrexed had a better response rate and longer PFS than the patients with wild-type EGFR. According to results reported by Camidge et al and Lee et al, pemetrexed therapy is associated with prolonged PFS in patients with NSCLC who have ALK rearrangements.

Many preclinical and clinical studies have demonstrated that DNA repair capacity and synthesis genes can correlate with clinical outcome in patients with NSCLC who receive chemotherapy. It has been postulated that impaired DNA repair capacity may be associated with increased genomic instability and increased tumor mutation rates. In preclinical studies, EGFR and DNA repair pathways have been linked, and these findings have implications for platinum-based therapy, possibly through BRCA1. Along this line, it has been demonstrated that cisplatin-induced cytotoxicity depends on EGFR-directed signaling, resulting in increased platinum-DNA adducts in high EGFR-expressing cell lines. Relatively little information exists on whether EGFR-mutant NSCLC cell lines are more sensitive to DNA-damaging chemotherapy or ionizing radiation in preclinical models. Das et al indicated that EGFR...
mutation enhanced sensitivity to radiation by a multifaceted process, including delayed DNA repair kinetics, suggesting that other components of the DNA repair mechanism may be involved in this association. Lee et al.\textsuperscript{33} detected ERCC1 expression by immunohistochemistry and EGFR mutations in resected NSCLC specimens and observed that EGFR mutation was associated significantly with lower ERCC1 expression levels. Furthermore, Gandara et al.\textsuperscript{34} investigated the correlation between EGFR mutation status and ERCC1 gene expression in patients with advanced NSCLC and reported that specimens harboring activating EGFR mutations were more likely to express low levels of ERCC1 mRNA. Consistent with those results, we observed that ERCC1 levels were significantly lower in the EGFR-mutated group in our population of women never-smokers with pulmonary adenocarcinoma. However, no difference was observed between the ALK rearrangement group and the negative group.

Pemetrexed is currently approved for the treatment of patients with nonsquamous cell histology as first-line treatment in combination with platinum,\textsuperscript{4} both as a second-line single agent\textsuperscript{35} and as maintenance therapy\textsuperscript{36} after first-line, platinum-based chemotherapy. Additional studies\textsuperscript{28,29} indicated that high TS expression is considered a resistance mechanism in NSCLC and may be a predictive biomarker of pemetrexed sensitivity. Furthermore, Lee et al.\textsuperscript{22} observed that TS mRNA levels were relatively low in ALK positive cells. Consistent with this result, in our current study, we also observed that patients with NSCLC who had ALK rearrangements were more likely to express low TS mRNA levels. In addition, patients with NSCLC who had EGFR mutations also were more likely to express low TS mRNA levels, which, to some extent, may explain the finding that EGFR mutation status also was a predictor of pemetrexed efficacy.\textsuperscript{27}

In our current study, no association was observed between EGFR mutation or ALK rearrangement status and the RRM1 and BRCA1 genes expression. Regarding these genes, few studies have investigated their relation to EGFR mutation or ALK fusion status. In an invivo study, Li et al.\textsuperscript{30} demonstrated a novel link between EGFR inhibitor and homology-directed recombinational repair and observed that BRCA1 status may affect the sensitivity of cancer cells to erlotinib in human breast cancer cells. In lung cancer, Rosell et al.\textsuperscript{37} investigated the predictive role of BRCA1 in the response to erlotinib for patients with EGFR mutations and observed that low BRCA1 levels were associated with longer PFS in response to erlotinib ($P = .02$). However, in our study, we did not observe a significant correlation between EGFR mutation and BRCA1 gene expression. Because BRCA1 plays an important role in the repair of erlotinib-induced DNA damage, mainly through the homologous recombination pathway,\textsuperscript{37} we hypothesize that the mechanism of BRCA1’s predictive role regarding the efficacy of erlotinib may be based on DNA repair ability and not on the association of its expression with EGFR mutation status, which may partly explain these discordant results.

In conclusion, we observed that mRNA expression levels of the ERCC1 and TS genes were associated with EGFR mutation and ALK rearrangement status. NSCLC specimens that harbor activating EGFR mutations are more likely to express low ERCC1 and TS mRNA levels, whereas tumors from patients who have NSCLC with ALK rearrangement are more likely to express low TS mRNA levels. Our findings support the hypothesis that EGFR mutations and ALK rearrangements affect the efficacy of chemotherapy through the pathways of DNA repair and synthesis genes. Because EGFR mutation and ALK rearrangement status will be established as a matter of routine before the initiation of any anticancer therapy in clinical practice, these 2 biomarkers also may be helpful in choosing the proper chemotherapy regimen for patients with NSCLC.

**FUNDING SOURCES**

This study was supported by Amoy Diagnostics Company Ltd., Xiamen, China and was supported in part by a grant from the National Science Foundation of China (81172108) and the key project of the Science and Technology Commission of Shanghai Municipality (No.06DZ19502).

**CONFLICT OF INTEREST DISCLOSURES**

The authors made no disclosures.

**REFERENCES**


