

Infrequent *ERBB2* mutations in Chinese patients with non-small cell lung cancer

Shuidong Feng^{1,2*}, Hongyan Ling^{3*}, Hui Guo⁴, Lingling Tong^{1,2}, Guobin Hu⁴, Li Liao^{1,2}, Xueying Lv^{1,2}, Hongzhan Tan⁵, Yimou Wu⁶

¹Basic Medical Postdoctoral Research Station, ²Department of Epidemiology & Health Statistics, School of Public Health, ³Department of Physiology, School of Medicine, University of South China, Hengyang 421001, China; ⁴The Pathology Department, Tumor Hospital of Hunan Province, Changsha 410008, China; ⁵Department of Epidemiology & Health Statistics, School of Public Health, Central South University, Changsha 410008, China; ⁶Department of Medical Microbiology, School of Medicine, University of South China, Hengyang 421001, China

*These authors contributed equally to the study.

Correspondence to: Hongzhan Tan. Department of Epidemiology & Health Statistics, School of Public Health, Central South University, 110 Xiangya Road, Kaifu District, Changsha 410008, China. Email: hongzhan@126.com; Yimou Wu. Department of Medical Microbiology, School of Medicine, University of South China, 28 West Changsheng Road, Zhenxiang District, Hengyang 421001, China. Email: yimowu@126.com.

Abstract: *ERBB2* mutations have been reported to occur in a subset of patients with lung adenocarcinomas or lung squamous cell carcinomas for some ethnicities, but it is unclear for Chinese patients with lung squamous cell carcinomas up to now. We retrospectively evaluated the status of *ERBB2* mutations in a large cross-sectional cohort of 212 Chinese patients with non-small cell lung cancer (NSCLC) diagnosed in several hospitals from southern China during a time period of 1.5 years by polymerase chain reaction (PCR)-based direct sequencing and PCR-single strand conformation polymorphism (PCR-SSCP) analysis. *ERBB2* mutation was found in 1 of 49 lung adenocarcinomas (2.0%) and none in lung squamous cell carcinomas and lung adenosquamous carcinomas. It implies the occurrence of *ERBB2* mutations is infrequent in Chinese patients with NSCLC, especially in lung squamous cell carcinomas.

Keywords: *ERBB2/HER2*; mutation; non-small cell lung cancer (NSCLC); molecular

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Introduction

Members of c-erb B family of oncogenes, which include epidermal growth factor receptor (*EGFR*, *ERBB1*), *ERBB2* (*HER2*), *ERBB3* (*HER3*), and *ERBB4* (*HER4*), play an important role in the development and progression of non-small cell lung cancer (NSCLC) by promoting cell growth and preventing apoptosis by regulating downstream effectors such as mitogen-activated protein kinase, protein kinase B, and signal transducer and activator of transcription 3 (1,2). Mutations in the kinase domain of *ERBB2* gene have been reported to occur in lung adenocarcinomas (3-5) and lung squamous cell carcinomas (6) for some ethnicities, which could potentially result in the activation of the tyrosine kinase activity of *ERBB2* protein and play a critical role in oncogenesis in a manner similar to *EGFR* mutations

(7,8). However, to date, it is unclear that the status of *ERBB2* mutations in Chinese patients with lung squamous cell carcinomas that are the majority of NSCLC.

To find out the overall status of *ERBB2* mutations in Chinese patients with NSCLC, in this study we conducted a detailed search for the *ERBB2* mutations in tumor tissues derived from a large cross-sectional cohort of Chinese patients with NSCLC who underwent tumor resection in several hospitals from southern China during a time period of 1.5 years.

Methods

Patients and tissue specimens

From June 2006 to November 2007, tumor tissues and

paired normal lung tissues were obtained from 212 Chinese patients with NSCLC who underwent curative resection in the Tumor Hospital of Hunan Province and the Xiangya Hospital and the Xiangya 2nd Hospital of Central South University which are top three hospitals in Hunan Province, China. None of these patients had received chemotherapy or radiotherapy before surgery. Written informed consent was obtained from each patient before the surgery. This study was approved by the institutional review boards respectively from the Tumor Hospital of Hunan Province and the Xiangya Hospital and the Xiangya 2nd Hospital of Central South University. Pathological histology type was determined according to WHO criteria (9). All of the tumor and macroscopically normal lung tissue samples were obtained at the time of surgery, and then they were rapidly frozen in liquid nitrogen and stored at -80°C . Only tumors with 80% or more tumor component were sent for DNA extraction and mutational analysis. The normal lung tissue specimens were obtained from either the opposite end of resected surgical samples or as distant as possible from the tumor. All of the macroscopically normal samples were confirmed as normal under hematoxylin and eosin staining.

Mutational analysis of *ERBB2* gene

Genomic DNA was obtained from frozen lung tissues by overnight digestion with sodium dodecyl sulfate and proteinase K [Tiangen Biotech (Beijing) Co., Ltd, China] at 37°C followed by standard phenole chloroform (1:1) extraction and ethanol precipitation. PCR-based direct sequencing and polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) were used to detect mutations of exons 19-20 of the TK domain of *ERBB2* gene. The PCR was performed in a total volume of $25\ \mu\text{L}$ containing 100 ng genomic DNA, 0.2 mmol/L of each primer, and 0.2 mmol/L dNTPs, 1 unit of Taq polymerase [Tiangen Biotech (Beijing) Co., Ltd, China], and 1 reaction buffer (10 mmol/L Tris-HCl, pH 8.3; 50 mmol/L KCl; and 1.5 mmol/L MgCl_2). The PCR cycle conditions consisted of an initial denaturation step at 95°C for 5 minutes followed by 30 cycles of 30 seconds at 95°C and 60 seconds at 72°C , and a final elongation at 72°C for 7 minutes. The primers used for exon 19 were: forward, 5'-TGG AGG ACA AGT AAT GAT CTC CTG G-3' and reverse, 5'-AAG AGA GAC CAG AGC CCA GAC CTG-3', amplifying a 160-bp fragment; and for exon 20 were: forward, 5'-GCC ATG GCT GTG GTT TGT GAT GG-3' and reverse, 5'-ATC CTA GCC CCT TGT GGA CAT

AGG-3', amplifying a 249-bp fragment. The PCR products were purified using a PCR Products Purification kit (Bio Basic Inc., CA), then sequencing was done using an ABI Prism 3100 Genetic Analyzer [Sangon Biotech (Shanghai) Co., Ltd, China]. Sequence chromatograms were analyzed by Mutation Surveyor 2.60, followed by manual review. At the same time, PCR-SSCP analysis was also performed for each sample. The purified PCR products were diluted 1:5 in loading buffer (95% formamide, 2 mM EDTA, pH 8.3), and then the diluted samples ($12\ \mu\text{L}$) were denatured (5 min at 90°C), immediately cooled on ice and loaded into a 12% non-denaturing polyacrylamide gel. Electrophoresis was carried out for 4 hr at 15°C at 5 Watt. Upon completing migration the gels were subject to staining using ethidium bromide for 20 minutes.

Results

Patient characteristics

Between June 2006 and November 2007, tumor specimens suitable for genetic analysis were available from 212 patients with NSCLC. The cohort included patients with age at diagnosis ranging from 41 to 77 (median age, 63) in 172 males and from 33 to 72 (median age, 57) in 40 females. There were 153 Squamous cell carcinomas (72.2%), 49 adenocarcinomas (23.1%) and 10 adenosquamous carcinomas (4.7%) in the cohort including 148 smokers (69.8%) and 64 never-smokers (30.2%).

The status of *ERBB2* mutation

The *ERBB2* mutation was found in 1 of 49 lung adenocarcinomas (2.0%), and none in 153 lung squamous cell carcinomas and 10 lung adenosquamous carcinomas. The single adenocarcinoma case with *ERBB2* mutation was a never-smoker, female and 44 years of age. The category of *ERBB2* mutation was a heterozygous in-frame insertion occurring within exon 20: 2327-2329insTTT (G776V, insC) (Figure 1). Likewise, PCR-SSCP analysis revealed no more than one *ERBB2* mutation within exon 20 in the study cohort. The mutated *ERBB2* presented two different electrophoresis bands distinguished from normal one (Figure 2).

Discussion

In the present study, we found only 1 *ERBB2* mutation in 49 lung adenocarcinomas (2.0%) and no *ERBB2* mutation

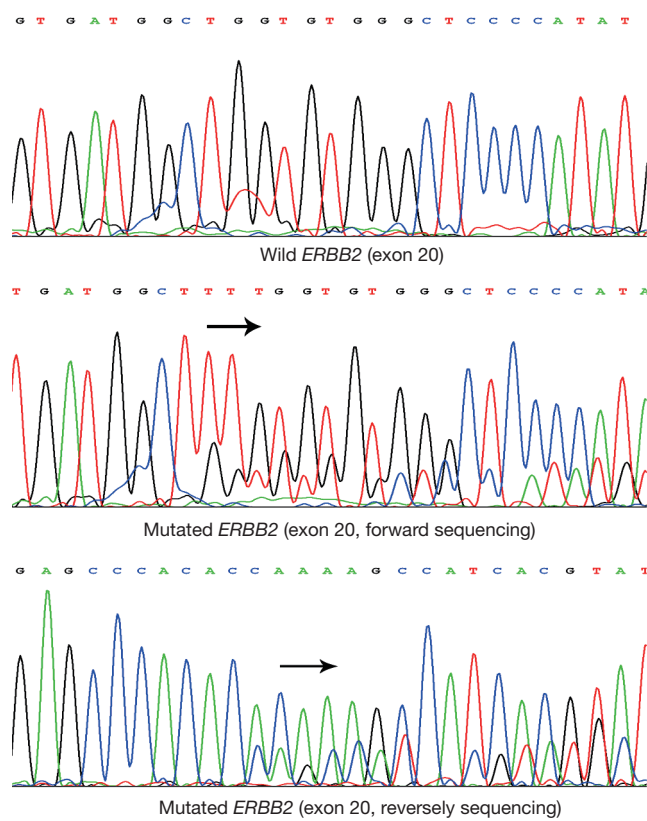


Figure 1 Sequencing map of wild and mutated *ERBB2* (exon 20).

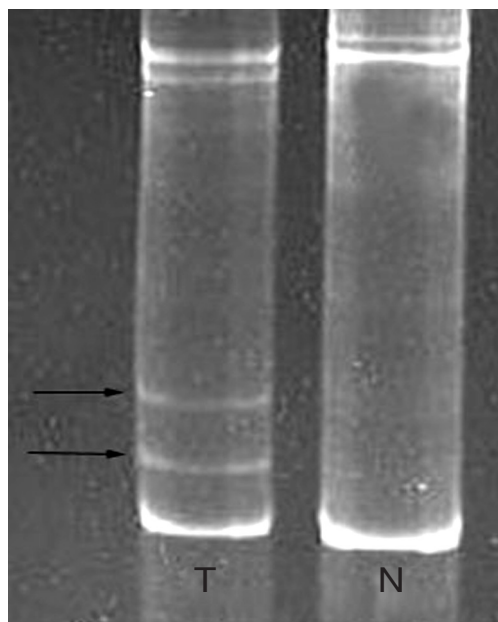


Figure 2 Electrophoretogram of mutated *ERBB2* by PCR-SSCP analysis (T, tumor tissue; N, normal tissue; Arrows, abnormal bands). PCR-SSCP, polymerase chain reaction-single strand conformation polymorphism.

in lung squamous cell carcinomas and lung adenosquamous carcinomas. The rate of *ERBB2* mutation in this study is lower than those of previous researches (10,11), which didn't involve lung squamous cell carcinomas and lung adenosquamous carcinomas. This difference may be due to the different distributions of sex and smoking status of study sample. In addition, the differences of environment and histological types should be considered too. Similar to our findings, Yokoyama *et al.* didn't find *ERBB2* mutations in Japanese lung squamous cell carcinomas (12).

As with *EGFR* mutations, *ERBB2* mutations, which may be a therapeutic target in lung cancer patients (13), are more common in Asians, adenocarcinomas, females, and never-smokers (4,5,14). The patient with *ERBB2* mutation in this study was never-smoker, female and adenocarcinomas. Thus, *ERBB2* mutations may play a key role in the development of adenocarcinomas in nonsmokers. A 3-bp in-frame insertion mutation within exon 20 in our patients is not the major type of mutation found by other investigators (3-5,13), which may be resulted from a small sized study sample, but it is consistent with those of Shigematsu and colleagues (4). Further investigation will be needed to clarify the relationship between *ERBB2* mutations and clinicopathologic features in Chinese patients with NSCLC.

In conclusion, our results suggest that *ERBB2* mutations occur infrequently in Chinese patients with NSCLC, especially in lung squamous cell carcinomas. So it is possible of limited value for molecular target therapy based on the mutated *ERBB2* in Chinese patients with NSCLC.

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