BRIEF REPORT

EGFR T790M and C797S Mutations as Mechanisms of Acquired Resistance to Dacomitinib

Yoshihisa Kobayashi, MD, PhD, Toshio Fujino, MD, Masaya Nishino, MD, Takamasa Koga, MD, Masato Chiba, MD, Yuichi Sesumi, MD, Shuta Ohara, MD, Masaki Shimoji, MD, Kenji Tomizawa, MD, PhD, Toshiki Takemoto, MD, PhD, Tetsuya Mitsudomi, MD, PhD*

Department of Thoracic Surgery, Kindai University Faculty of Medicine, Osaka-Sayama, Japan

Received 27 December 2017; revised 11 January 2018; accepted 14 January 2018
Available online - 2 February 2018

ABSTRACT

Introduction: Dacomitinib was superior to gefitinib in terms of progression-free survival in patients with *EGFR*-mutant lung cancer in a recent ARCHER 1050 trial. However, despite a marked initial response, lung cancers eventually acquire resistance to these inhibitors. This study aimed to elucidate the mechanisms of acquired resistance to dacomitinib in vitro.

Methods: Dacomitinib-resistant clones were established by exposure to fixed concentrations of dacomitinib by using *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis or by chronic exposure to increasing concentrations of dacomitinib without ENU. *EGFR* secondary mutations were analyzed by Sanger sequencing. Time to resistance in each clone was compared according to the mutational status. *EGFR* Del19, L858R, and G719A mutations were introduced into Ba/F3 cells by using retroviral vectors.

Results: Chronic exposure to dacomitinib without ENU induced T790M in Ba/F3 cells expressing Del19. ENU mutagenesis resulted in 171 dacomitinib-resistant clones. Among these clones, 90% acquired T790M. However, C797S occurred in 11% of L858R-mutant clones (four of 35) and in 24% of G719A-mutant clones (12 of 38) established by using low-dose dacomitinib. Time to resistance was not significantly different between T790M- and C797S-mutant clones in both of L858R clones ($p = 0.93$) and G719A clones ($p = 0.86$). Cells expressing Del19 that acquired T790M were sensitive to osimertinib, whereas cells with L858R plus C797S mutations were sensitive to gefitinib or erlotinib.

Conclusions: These in vitro data demonstrate that dacomitinib can directly induce T790M or C797S secondary mutations. Our data suggest the importance of analyzing these secondary mutations because appropriate selection of EGFR inhibitors could overcome acquired resistance to dacomitinib in a subset of lung cancers.

Keywords: Adenocarcinoma; Dacomitinib; *EGFR* mutation; Lung cancer; Tyrosine kinase inhibitor

Introduction

Targeted therapy with tyrosine kinase inhibitors (TKIs) is a standard treatment for patients with epidermal growth factor receptor gene (*EGFR*)-mutated lung cancer. The ARCHER 1050 trial demonstrated superiority of dacomitinib, an irreversible second generation (2G) TKI, to reversible first-generation (1G) gefitinib in terms of progression-free survival (PFS).[1] The median PFS times were 14.7 months in the dacomitinib group and 9.2 months in the gefitinib group, with a hazard ratio of 0.59.[1]

Despite the marked initial response, lung cancers inevitably acquire resistance to these TKIs. The major

*Corresponding author.

Disclosure: Dr. Mitsudomi has received lecture fees from AstraZeneca, Boehringer Ingelheim, Chugai, and Pfizer and research funding from Astra Zeneca, Boehringer Ingelheim, and Chugai during the conduct of the study. Dr. Mitsudomi has been an advisory board of Novartis and has received lecture fees from Bristol-Myers Squibb, Eli Lilly, Merck Sharp and Dohme, and Taiho and research funding from Daiichi Sankyo, Taiho, and Ono Pharmaceutical outside the submitted work. Dr. Kobayashi has received a lecture fee from Boehringer Ingelheim and research funding from Novartis during the conduct of the study. The remaining authors declare no conflict of interest.

Address for correspondence: Tetsuya Mitsudomi, MD, PhD, Department of Thoracic Surgery, Kindai University Faculty of Medicine, 377-2 Ohno-Higashi, Osaka-Sayama, 589-8511, Japan. E-mail: mitsudom@med.kindai.ac.jp

ISSN: 1556-0864

https://doi.org/10.1016/j.jtho.2018.01.009

mechanism of acquired resistance to gefitinib, erlotinib, or afatinib is known to be a secondary *EGFR* T790M mutation,[2] which can be overcome by osimertinib.[3] However, except for data on bypass tracks and *EGFR* amplifications, data on the mechanisms of acquired resistance to dacomitinib are limited.[4–6] To overcome resistance and discuss the best sequence of TKI therapy, it is essential to elucidate the associated resistance mechanisms.

The purpose of this study was to elucidate *EGFR* secondary mutations as mechanisms of acquired resistance to dacomitinib using in vitro models with or without *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis.

Materials and methods

Cell Culture and Reagents

The interleukin-3–dependent murine pro–B-cell line Ba/F3 was provided by RIKEN Bio Resource Center (Tsukuba, Japan). Cells were cultured in Roswell Park Memorial Institute 1640 medium (Wako, Osaka, Japan) with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO). EGFR TKIs (gefitinib, erlotinib, dacomitinib, and osimertinib) were purchased from Selleck Chemicals (Houston, TX).

Construction of Retroviral Vector–Transduced Cell Lines

EGFR mutations were introduced into Ba/F3 cells by using retroviral vectors as previously described.[7,8] Briefly, pBABE retroviral vector with the full-length complementary DNA encoding *EGFR* gene was provided by Addgene (Cambridge, MA). The *EGFR* Del19 (del E746_A750), L858R, G719A, and L858R plus C797S mutations were generated by using a Prime-STAR Mutagenesis Basal Kit (Takara, Kusatsu, Japan). The constructs were cotransfected with a pVSV-G vector (Clontech, Palo Alto, CA) into gpIRES-293 cells using FuGENE6 transfection reagent (Roche Diagnostics, Basel, Switzerland) to produce viral particles. Infected Ba/F3 cells were cultured in the absence of interleukin-3.

Establishment of Dacomitinib-Resistant Clones

We previously demonstrated that the concentrations for 90% inhibition of Ba/F3 cells transfected with Del19, L858R, or G719A were lower than 6 nM.[7] Considering that the clinically achievable trough concentration (the lowest concentration reached by a drug before the next dose is administered) of dacomitinib was 166 nM in a phase I trial,[9] these three mutations seem to be sensitive to dacomitinib. Therefore, these three cells were used in the present study.

Dacomitinib-resistant clones were rapidly established by ENU (Sigma Aldrich) mutagenesis.[8] After the exposure to 100 μg/mL of ENU for 24 hours, cells were washed with Roswell Park Memorial Institute medium and cultured for 24 hours. Between 1×10^4 and 1×10^5 cells were plated in 96-well plates in the presence of dacomitinib. The concentration of dacomitinib was set to 200 nM considering the above-trough concentration.[9] Additionally, a low concentration (20 nM) of dacomitinib was also evaluated to mimic the dose modification in the clinic. When the cells reached confluence, they were regarded as having acquired resistance.

In another experiment, Ba/F3 cells expressing Del19 were chronically exposed to increasing concentrations of dacomitinib. The final concentration of dacomitinib was set at 200 nM.

EGFR *Mutational Analyses*

Total RNA was isolated from resistant cells and then reverse-transcribed into complementary DNA. *EGFR* exons 18 to 21 were amplified with primers, and Sanger sequencing was performed by using a Genetic Analyzer 3130 or 3500XL (Applied Biosystems, Waltham, MA) as previously reported.[8]

Statistical Analyses

Time to resistance to dacomitinib was defined as the time between the initial date of exposure to dacomitinib and the date on which the cells reached confluence. Time-to-resistance curves according to each *EGFR* secondary mutation were estimated by the Kaplan-Meier method, and differences were compared by using the log-rank test by JMP, version 8.0.2 (SAS Institute Inc., Cary, NC).

Cell Growth Inhibition Assay

A quantity of 2×10^3 cells were plated and grown for 24 hours. After 72 hours of exposure to EGFR TKIs, Cell Counting Kit-8 reagent (Dojindo Laboratories, Kumamoto, Japan) was added. The absorbance at 450 nm was read with a multiplate reader (Tecan, Mannedorf, Switzerland). The data are expressed as growth percentages relative to that of the DMSO-treated controls.

Results

Secondary EGFR *Mutations in Dacomitinib-Resistant Clones*

To clarify secondary *EGFR* mutations as resistance mechanisms and the frequency of these mutations, we generated resistant clones through ENU mutagenesis. In total, 171 resistant clones were obtained (Fig. 1). T790M mutations accounted for 90% of the secondary mutations (154 of 171). C797S mutations arose in L858R-mutant cells (four of 35) and G719A-mutant cells (12 of 50) in the low-dose (20 nM) setting. One G719A-mutant clone developed C797S even with the high dose of dacomitinib.