Lung cancers with acquired resistance to EGFR inhibitors occasionally harbor BRAF gene mutations but lack mutations in KRAS, NRAS, or MEK1

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AUTHOR SUMMARY

Lung cancers with somatic epithelial growth factor receptor (EGFR) mutations initially are highly sensitive to the EGFR tyrosine kinase inhibitors (TKIs) gefitinib or erlotinib (1), but progression of disease (i.e., “acquired” or “secondary” resistance) occurs after about a year. In up to 40% of patients, mechanisms of resistance are unexplained. Here, through analysis of nearly 200 tumor samples for known “hotspot” mutations in genes (KRAS, NRAS, MEKI, or BRAF) encoding components of the EGFR signaling pathway (Fig. P1), we report that mutations in BRAF mediate resistance in 1% of cases. These results provide deeper insights into mechanisms of acquired resistance, inform ongoing clinical trials designed to treat refractory disease, and suggest that, among these genes, only BRAF mutations need be determined routinely in samples from patients.

Mechanisms of resistance to TKIs that have been revealed by studies of EGFR-mutant lung adenocarcinomas include second-site resistance EGFR mutations (>50%), amplification of the gene encoding the receptor MET (5–10%), mutations in the gene (PIK3CA) encoding the p110α catalytic subunit of the downstream signaling lipid kinase PI3K (<5%), and histologic transformation [i.e., cells display epithelial-mesenchymal transition (EMT) or small cell lung cancer (<5%)]. Here, to explore other potential modes of drug resistance, we used five TKI-sensitive parental human EGFR-mutant lines to develop six lines with acquired resistance. Five of six cells developed known mechanisms, i.e., an EGFR T790M mutation, MET amplification, or EMT. One cell line (11-18R) displayed an unexpected acquired NRAS Q61K mutation. Resistant cells were sensitive to concurrent treatment with EGFR and MEK inhibitors but to neither drug alone. Consistent with these findings, only the combination of erlotinib and an MEK inhibitor strongly diminished levels of phospho-ERK, a signaling protein that acts downstream of NRAS in the EGFR signaling pathway.

Mutations in KRAS, NRAS, MEKI, or BRAF now have

Fig. P1. Cell-proliferative signaling pathways in lung tumors. EGFRs signal through the PI3K/AKT and RAS/MEK/ERK cascades. (i) WT EGFRs are activated in a ligand-dependent manner. (ii) Mutant EGFRs are constitutively activated. EGFR TKIs block kinase activity from mutant EGFR and inhibit downstream signaling. (A and D) Acquisition of NRAS or BRAF mutations (mts) leads to constitutive activation of the RAS/MEK/ERK pathway. (B and E) EGFR-TKIs inhibit EGFR, but mutant NRAS or BRAF maintain downstream ERK activation. (C) The combination of EGFR and MEK inhibitors can overcome resistance caused by the acquisition of NRAS mutations in EGFR-mutant cells. (F) The combination of EGFR and BRAF inhibitors or EGFR and MEK inhibitors can overcome resistance caused by the acquisition of BRAF mutations in EGFR-mutant cells.


Conflict of interest statement: L.V.S. received consulting fees from Clovis Oncology, GlaxoSmithKline, and Celgene Corporation. J.C.-H.Y. received consulting fees from Boehringer Ingelheim. V.A.M. is an employee at Foundation Medicine and has equity value in the company. M.G.K. has received consulting fees from Boehringer Ingelheim and research funding for other projects from Pfizer and Boehringer. J.A.E. has received consulting fees and has stock option ownership in Aqios Pharmaceuticals and has received research funding for other projects from Novartis, GlaxoSmithKline, and AstraZeneca. D.D.S. received consulting fees from Bio-Reference Laboratories. W.P. has received consulting fees from MolecularMD, AstraZeneca, Bristol-Myers Squibb, Symphony Evolution, and Clovis Oncology and research funding for other projects from Enzon, Ksyvery, AstraZeneca, and Symphogen. W.P. and V.A.M. are part of a patent regarding EGFR T790M mutation testing that was licensed by Memorial Sloan-Kettering Cancer Center to MolecularMD.

This article is a PNAS Direct Submission. Freely available online through the PNAS open access option.

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See full research article on page E2126 of www.pnas.org.

Cite this Author Summary as: PNAS 10.1073/pnas.1203530109.
emerged as mediators of acquired resistance to targeted therapies in a variety of cancers. In colorectal cancers, KRAS mutations are associated with resistance to the anti-EGFR monoclonal antibody cetuximab. In melanomas, NRAS and MEK1 mutations mediate resistance to the inhibitor of mutant BRAF kinase, vemurafenib. In gastrointestinal stromal tumors (GISTs) harboring mutations in genes encoding other members of the protein tyrosine kinase receptor superfamily (KIT and PDGFRα), BRAF mutations occur in patients after long-term treatment with imatinib. KRAS mutations are not detected in lung tumors from patients with secondary resistance to EGFR-TKIs (2–4). However, the sample sizes are small (n = 37, 14, and 6, respectively, in three studies). Only one study examined samples for BRAF mutations; no studies examined MEK1.

Prompted by these data and by the finding that a clinically relevant mouse lung tumor model of acquired resistance to TKIs also identified secondary Kras mutations (5), we systematically analyzed the frequency of known hotspot mutations in KRAS, NRAS, MEK1, and BRAF in the largest known collection of samples from patients with acquired TKI resistance. No recurrent NRAS, KRAS, or MEK1 mutations were detected in 212, 195, or 146 patient samples, respectively, but one tumor simultaneously harbored the mutations as follows: EGFR exon19 deletion, EGFR T790M, and BRAF V600E; another harbored an EGFR exon19 deletion with BRAF G469A. Ectopic expression of mutant NRAS or BRAF in drug-sensitive EGFR-mutant cells conferred resistance to EGFR TKIs. In stable transfectants with mutant NRAS or BRAF, the combination of erlotinib and an MEK inhibitor significantly inhibited cell growth and reduced the levels of phospho-ERK (Fig. P1). In the case of BRAF V600E, erlotinib plus vemurafenib had the same effect as an MEK inhibitor.

Targeted therapies are being developed at a rapid pace for various cancers. The data presented here further highlight the notion that, even though colorectal cancers, melanomas, GISTs, and lung cancers share common cell-proliferative signaling cascades, mediators of resistance must be elucidated separately for each disease to design individualized treatment.