IN THE SPOTLIGHT

CRKL as a Lung Cancer Oncogene and Mediator of Acquired Resistance to EGFR Inhibitors: Is It All That It Is Cracked Up to Be?

Marc Ladanyi

Summary: Cheung and colleagues demonstrate that amplified CRKL can function as a driver oncogene in lung adenocarcinoma, activating both RAS and RAP1 to induce mitogen-activated protein kinase signaling. In addition, they show that CRKL amplification may be another mechanism for primary or acquired resistance to epidermal growth factor receptor kinase inhibitors. *Cancer Discovery; 1(7);* 560–1. *©2011 AACR.*

Commentary on Cheung et al., p. 608 (1).

In this issue of *Cancer Discovery*, Cheung and colleagues (1) present extensive genomic and functional data supporting focal amplification of the *CRKL* gene at 22q11.21 as a driver oncogene in lung adenocarcinoma. The report expands on data previously presented by Kim and colleagues (2). CRKL is a signaling adaptor protein that contains SH2 and SH3 domains that mediate protein–protein interactions linking tyrosine-phosphorylated upstream signaling molecules (e.g., BCAR1, paxillin, GAB1) to downstream effectors (e.g., C3G, SOS) (3). Cheung and colleagues demonstrate that overexpressed CRKL induces the transformation of human lung epithelial cells through activation of both RAS and RAP1, resulting in robust activation of the mitogen-activated protein kinase pathway. *CRKL* was first nominated as a possible driver of 22q11 gains in lung cancer in 2005 (4). Subsequently, focal amplification of *CRKL* has been confirmed as an uncommon but consistent finding in lung adenocarcinoma.

Cheung and colleagues (1) report a prevalence of 3% in tumors (on the basis of their previous data; ref. 5) and 7% (6/84) in cell lines. This finding is similar to other independent series, including that of Chitale and colleagues (6), who noted narrow amplicons encompassing *CRKL* in 6% of lung adenocarcinomas, and that of Kim and colleagues (2), who reported a frequency of 3%. In addition, approximately 2- to 3-fold more cases harbor broader gains of 22q; the CRKL dependence of such tumors will also be important to assess because it would impact on the size of the patient subset in terms of future targeted clinical approaches.

Is amplified *CRKL* a driver oncogene of the same rank or stature as mutant epidermal growth factor receptor (*EGFR*)? One of the notable features of major driver oncogenes (*EGFR*, *KRAS*, *HER2*, *BRAF*) in lung adenocarcinoma is their mutual exclusivity. Cheung and colleagues (1) report that focal *CRKL* amplification is mutually exclusive with *EGFR* mutation and *EGFR* amplification. However, of the 6 lung cancer cell lines found in this study to have focal gains of *CRKL*, 2 contain other major driver oncogenes (*KRAS* G13D in HCC515, *BRAF* G469A in H1755; refs. 7, 8). Interestingly, both cell lines demonstrated clear dependence on CRKL in functional assays. Perhaps *CRKL* amplification is more akin to *PIK3CA* mutations, which often, but not always, are concurrent with other major driver oncogenes (9). Intriguingly, of the same 6 cell lines, at least 4 are known to have inactivating mutations in *LKB1*(7), suggesting another potential cooperating interaction to explore functionally. The investigators do provide functional evidence for another potentially important cooperating lesion, namely loss of *NF1*, and go on to show that 1 of 3 *CRKL*-amplified tumors also harbored an inactivating mutation of *NF1* (1). Clearly, the cooperative effects of *CRKL* gain and overexpression on various oncogenic lesions in these signaling pathways will require further work.

More broadly, the findings of Cheung and colleagues heighten the potential interest of *CRKL* gains in other cancers and of gains of other signaling adaptor molecules. In a survey of genomic copy number data on more than 3,000 specimens from 26 types of cancer, Beroukhim and colleagues (10) found *CRKL* at the epicenter of 1 of the top 12 most commonly amplified regions in multiple cancer types, including lung cancers, melanoma, ovarian cancer, and colorectal cancer. More generally, these investigators also found that regions of statistically significant gain across different cancers were significantly enriched for genes associated with the Gene Ontology term molecular adaptor activity (10). In addition to *CRKL*, these genes included *IRS2*, *GRB2*, *GRB7*, *GAB2*, and *TRAF6*, among others. Like CRKL, several of these have been shown to have oncogenic properties when gained or overexpressed, for instance, IRS2 and TRAF6 (11, 12).

Finally, could secondary amplification of *CRKL* represent yet another mechanism of acquired resistance to EGFR kinase inhibitors? Cheung and colleagues show that overexpression of CRKL decreases sensitivity to the EGFR inhibitor gefitinib in experiments based on introducing a *CRKL* expression

Author's Affiliations: Department of Pathology and Human Oncology and Pathogenesis Program, Memorial Sloan-Kettering Cancer Center, New York, New York

Corresponding Author: Marc Ladanyi, Department of Pathology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10065. Phone: 212-639-6369; Fax: 212-717-3515; E-mail: ladanyim@mskcc.org

doi: 10.1158/2159-8290.CD-11-0295

plasmid into the gefitinib-sensitive, EGFR-mutant HCC827 cell line (1). It will be of interest to see whether secondary amplification of *CRKL* ever emerges spontaneously after long-term selection of *EGFR* mutant cell lines in the presence of EGFR inhibitor, like the 2 major mechanisms of resistance, the *EGFR* T790M mutation and *MET* amplification (13–15). The spectrum of acquired resistance mechanisms for EGFR inhibitors has recently been more accurately defined by 2 large series in which the authors analyzed rebiopsy specimens from patients who progressed (16, 17). When high-sensitivity assays are used, the EGFR T790M or other rare second-site mutations are detected in 60% to 70% of patients (16). Another 10% of cases show acquired *MET* amplification, small cell transformation, or epithelial–mesenchymal transition (17), leaving approximately 25% to 30% of cases in which the precise mechanism of acquired resistance remains unknown.

In this context, it is notable that Cheung and colleagues also report the identification of 1 patient with acquired resistance to an EGFR inhibitor whose rebiopsy specimen showed a modest gain in *CRKL* copy number, possibly attributable to chromosome 22 polysomy, relative to the pretreatment baseline sample. Thus, it will be important to examine additional acquired resistance samples for such gains and to define their relationship to *EGFR* T790M. Likewise, it will be of interest to assess the status of *CRKL* in tumor biopsies from patients with *EML4-ALK*–positive lung cancer presenting acquired resistance to crizotinib, especially those lacking *ALK* mutations (18–20), because the biology of CRKL-induced resistance should in principle also apply to this subset. It is increasingly clear that the delineation of molecular subsets of lung cancer has dramatically clarified its biologic and clinical heterogeneity, leading to new therapeutic opportunities (21); the elucidation of the subset of lung cancers with focal *CRKL* amplification represents a further advance in this direction.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Received November 10, 2011; revised November 10, 2011; accepted November 10; published online December 14, 2011.

REFERENCES

1 Cheung HW, Du J, Boehm JS, He F, Weir BA, Wang X, et al. Amplification of CRKL induces transformation and EGFR inhibitor resistance in human non small cell lung cancers. Cancer Discovery 2011;1:608–25.

2 Kim YH, Kwei KA, Girard L, Salari K, Kao J, Pacyna-Gengelbach M, et al. Genomic and functional analysis identifies CRKL as an oncogene amplified in lung cancer. Oncogene 2010;29:1421–30.

3 Feller SM. Crk family adaptors-signalling complex formation and biological roles. Oncogene 2001;20:6348–71.

4. Zhao X, Weir BA, LaFramboise T, Lin M, Beroukhim R, Garraway L, et al. Homozygous deletions and chromosome amplifications in human lung carcinomas revealed by single nucleotide polymorphism array analysis. Cancer Res 2005;65:5561–70.
5. Weir BA, Woo MS, Getz G, Perner S, Ding L, Beroukhim R, et al. Characterizing the cancer genome in lung adenocarcinoma. Nature 2007;450:893–8.
6. Chitale D, Gong Y, Taylor BS, Broderick S, Brennan C, Somwar R, et al. An integrated genomic analysis of lung cancer reveals loss of DUSP4 in EGFR-mutant tumors. Oncogene 2009;28:2773–83.
7. Blanco R, Iwakawa R, Tang M, Kohno T, Angulo B, Pio R, et al. A gene-alteration profile of human lung cancer cell lines. Hum Mutat 2009;30:1199–206.
8. Pratilas CA, Hanrahan AJ, Halilovic E, Persaud Y, Soh J, Chitale D, et al. Genetic predictors of MEK-dependence in non-small cell lung cancer. Cancer Res 2008;68:9375–83.
9. Chaft JE, Arcila ME, Paik PK, Lau C, Riely GJ, Pietanza MC, et al. Coexistence of PIK3CA and other oncogene mutations in lung adenocarcinoma—rationale for comprehensive mutation profiling. Mol Cancer Therapeut, in press.
10. Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, et al. The landscape of somatic copy-number alteration across human cancers. Nature 2010;463:899–905.
11. Starczynowski DT, Lockwood WW, Delehouzee S, Chari R, Wegrzyn J, Fuller M, et al. TRAF6 is an amplified oncogene bridging the RAS and NF-kappaB pathways in human lung cancer. J Clin Invest 2011;121:4095–105.
12. Dearth RK, Cui X, Kim HJ, Hadsell DL, Lee AV. Oncogenic transformation by the signaling adaptor proteins insulin receptor substrate (IRS)-1 and IRS-2. Cell Cycle 2007;6:705–13.
13. Ogino A, Kitao H, Hirano S, Uchida A, Ishiai M, Kozuki T, et al. Emergence of epidermal growth factor receptor T790M mutation during chronic exposure to gefitinib in a non small cell lung cancer cell line. Cancer Res 2007;67:7807–14.
14. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007;316:1039–43.
15. Chmielecki J, Foo J, Oxnard GR, Hutchinson K, Ohashi K, Somwar R, et al. Optimization of dosing for EGFR-mutant non-small cell lung cancer with evolutionary cancer modeling. Sci Transl Med 2011;3:90ra59.
16. Arcila ME, Oxnard GR, Nafa K, Riely GJ, Solomon SB, Zakowski MF, et al. Rebiopsy of lung cancer patients with acquired resistance to EGFR inhibitors and enhanced detection of the T790M mutation using a locked nucleic acid-based assay. Clin Cancer Res 2011;17:1169–80.
17. Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 2011;3:75ra26.
18. Choi YL, Soda M, Yamashita Y, Ueno T, Takashima J, Nakajima T, et al. EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. N Engl J Med 2010;363:1734–9.
19. Sasaki T, Koivunen J, Ogino A, Yanagita M, Nikiforow S, Zheng W, et al. A novel ALK secondary mutation and EGFR signaling cause resistance to ALK kinase inhibitors. Cancer Res 2011;71:6051–60.
20. Katayama R, Khan TM, Benes C, Lifshits E, Ebi H, Rivera VM, et al. Therapeutic strategies to overcome crizotinib resistance in non-small cell lung cancers harboring the fusion oncogene EML4-ALK. Proc Natl Acad Sci U S A 2011;108:7535–40.
21. Pao W, Iafrate AJ, Su Z. Genetically informed lung cancer medicine. J Pathol 2011;223:230–40.

CANCER DISCOVERY

CRKL as a Lung Cancer Oncogene and Mediator of Acquired Resistance to EGFR Inhibitors: Is It All That It Is Cracked Up to Be?

Marc Ladanyi

Cancer Discovery 2011;1:560-561.