A driving test for oncogenic mutations

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Activating mutations in protein kinases are a frequent cause of cancer, and selecting drugs that act on these oncogenic kinases can lead to effective therapies. Targeted or whole-genome sequencing of tumor samples can readily reveal the presence of mutations, but discerning previously uncharacterized activating "driver" mutations that will respond to drug treatment from much more abundant but inconsequential "passenger" mutations is problematic. Chakroborty *et al.* apply a screening approach that leverages error-prone PCR and a proliferating cell model to identify such gain-of-function mutants in the epidermal growth factor receptor (EGFR) kinase. The screen is validated by the identification of known cancer-promoting mutations and reveals a previously unappreciated oncogenic EGFR mutation, A702V, demonstrating its power for discovery of driver mutations.

Altered proliferation without regard to normal cell and tissue contextual signals is a hallmark of cancer, and acquisition of mutations in selected growth factor receptors that activate signaling networks is a common route to tumorigenesis (1). Identifying the critical mutations that drive the growth of a particular cancer can lead to more specific and effective treatments. However, cancers generally accumulate mutations as a result of genome instability and high mutation rates, and the causative driver mutations are rare relative to the numbers of apparently inconsequential passenger mutations (2). Even in growth factor receptor kinases with known oncogenic potential, driver mutations may hide among the passengers. This "needle-in-the-haystack" situation complicates the identification of novel gain-of-function mutations that might indicate that a tumor will respond to an available targeted therapy, such as an epidermal growth factor receptor (EGFR)[2] tyrosine kinase inhibitor (TKI). How then can we accelerate the examination of mutated variants in proteins with a causative role in cancer phenotypes?

A new study by Chakroborty *et al.* (3) offers a solution with the development and application of a screening approach that enables rapid discernment of activating mutations in receptor tyrosine kinases. They apply their screen to detect functionally significant activating mutations within EGFR, a well-characterized receptor tyrosine kinase known to harbor diverse activating mutations in a variety of cancers and where such mutations are predictive for effective clinical responses with EGFR TKIs (4, 5). Starting with WT EGFR DNA, random mutations were introduced via error-prone PCR to generate a pool of single-nucleotide variants (SNVs), which were subsequently inserted into a retroviral mammalian expression vector (Fig. 1*A*). To discern driver *versus* nondriver mutations, the authors take advantage of Ba/F3 cells, a murine lymphoid cell line model that is routinely employed in the study of kinase oncoproteins (6). Whereas Ba/F3 cells readily proliferate in the presence of exogenous interleukin-3 (IL-3), their dependence on IL-3 can be overridden if they are transfected with an activated tyrosine kinase such as EGFR bearing an activating mutation. Thus, the authors use retroviral transduction to introduce the pool of EGFR variants into Ba/F3 cells and select for those that are activating by withdrawing IL-3. Targeted next-generation sequencing methods are then used to identify EGFR SNVs that are enriched in the surviving cell population as compared with the initial pool.

Remarkably, this mutational strategy allowed evaluation of more than 7000 unique EGFR variants, constituting 85% of all possible EGFR variants with an altered amino acid caused by a single nucleotide change in EGFR. More exhaustive mutagenesis strategies have been devised, such as mutagenesis by integrated tiles (MITE-Seq), which allows mutation of every residue of a protein of interest to each of the 19 other amino acids (7). However, the approach employed in this study is technically simpler and focuses on amino acid substitutions that are accessible via single nucleotide changes, which constitute the vast majority of cancer driver mutations. Indeed, the mutant pool deployed in this study covered all such mutations in EGFR, and the Ba/F3 selection approach also identified 21 SNVs that were enriched upon IL-3 withdrawal. Importantly, the well-established L858R activating point mutation was among the 21 mutants identified, confirming the power of the screen to identify clinically relevant mutations. In addition, the screen identified the T790M point mutation, a common treatment-acquired mutation that confers resistance to first-generation EGFR TKIs and is also known to oncogenically activate the kinase (8).

More interestingly, this approach simultaneously revealed a previously uncharacterized activating mutation in EGFR, A702V. Alanine 702 lies near the N terminus of the EGFR kinase domain and is part of the "asymmetric dimer" interface

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[2] The abbreviations used are: EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; SNV, single-nucleotide variant; IL-3, interlukin-3.

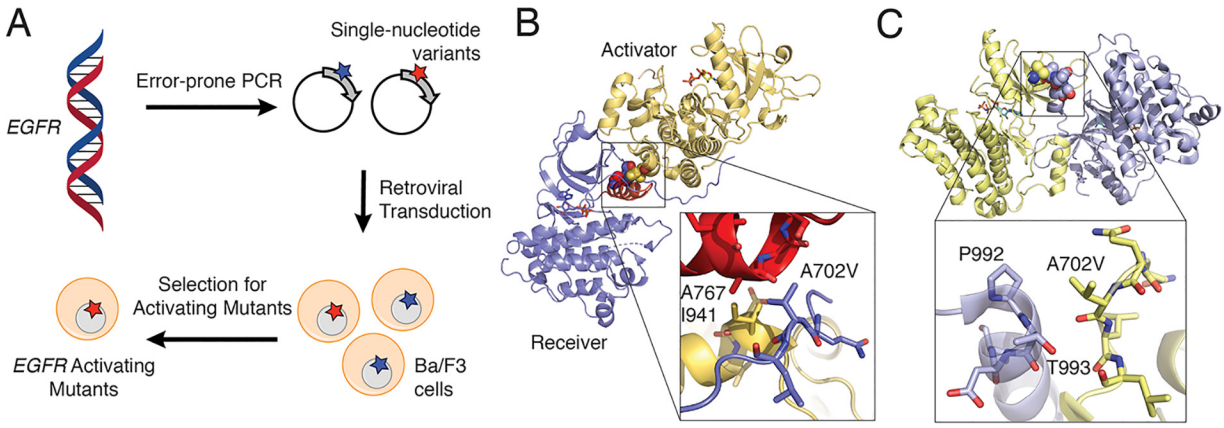

Figure 1. Identification of novel EGFR-activating mutations. *A*, screening workflow for identifying *EGFR* activating mutations. Single-nucleotide variants are generated by amplifying WT *EGFR* with error-prone PCR followed by cloning and retroviral transduction of the random DNA mutations into Ba/F3 cells. Activating mutations are selected by withdrawing IL-3, and surviving cells are sequenced. This method revealed well-known *EGFR* mutations as well as a novel A702V activating mutation postulated to reinforce the active asymmetric EGFR kinase dimer. *B*, molecular model generated with active EGFR crystal structures (Protein Data Bank code 2GS6) shows activator (*yellow*) and receiver (*blue*; αC-helix in *red*) kinase domains and the A702V site *highlighted* in *spheres*. The *inset* shows the proximity of A702V to hydrophobic residues (Ile-941 of activator and Ala-767 from αC-helix of the receiver) enabling more favorable binding of the two kinase domains. *C*, the A702V mutation is modeled in the crystal structure of a symmetric, inactive EGFR kinase dimer (Protein Data Bank code 3GT8). The two kinase domains are shown in *blue* and *yellow*, and the *inset* shows how the A702V mutation may introduce steric clashes with the C-terminal helix of the opposing kinase domain that weaken the inactive dimer.

that is the key to normal, ligand-driven EGFR activation (9). The authors hypothesize that the A702V mutation may lead to EGFR activation by promoting formation of this dimer (Fig. 1*B*). Their structural modeling and molecular dynamics simulations indicate that substitution of alanine with valine at this position stabilizes the active dimer via enhanced hydrophobic interactions in the interface and may thereby promote activation of EGFR in the absence of typical growth factor-dependent dimerization. An additional possibility, not discussed by Chakroborty *et al.*, is that the A702V mutation may *destabilize* an autoinhibited EGFR dimer to promote conversion to the active state. In the inactive state, the EGFR kinase domain is known to form a symmetric dimer that is thought to represent a quiescent state of the receptor (10). The crystal structure of the inactive EGFR dimer shows that Ala-702 lies in the dimer interface, juxtaposed with Pro-992 and Thr-993 of the other kinase domain in the dimer (Fig. 1*C*). Hence, the A702V mutation may introduce steric clashes that destabilize the inactive dimer. These two mechanisms are not mutually exclusive; both may contribute to ligand-independent EGFR activation. The authors also note that the A702V mutation has been observed previously in a non-small-cell lung cancer patient who exhibited a partial response to the EGFR TKI erlotinib; Chakraborty *et al.* now report biochemical characterization of this mutant, providing an explanation as to why it might not have responded well to erlotinib and showing that it is very sensitive to afatinib, an irreversible EGFR TKI.

The screening approach reported by Chakroborty *et al.* (3) is applicable to essentially any receptor tyrosine kinase that can support IL-3–independent growth of Ba/F3 cells and therefore has the potential to accelerate the identification of driver mutations in other kinase oncogenes. For well-established oncogenic kinases for which targeted therapies are available, correct classification of rare variants identified in tumor biopsies as driver mutations that are likely to contribute to the response of cancer cells to a targeted therapy is crucial for rational clinical decision-making.

References

1. Hanahan, D., and Weinberg, R. A. (2011) Hallmarks of cancer: the next generation. *Cell* **144,** 646–674 CrossRef Medline
2. Pon, J. R., and Marra, M. A. (2015) Driver and passenger mutations in cancer. *Annu. Rev. Pathol.* **10,** 25–50 CrossRef Medline
3. Chakroborty, D., Kurppa, K. J., Paatero, I., Ojala, V. K., Koivu, M., Tamirat, M. Z., Koivunen, J. P., Jänne, P. A., Johnson, M. S., Elo, L. L., and Elenius, K. (2019) An unbiased *in vitro* screen for activating epidermal growth factor receptor mutations. *J. Biol. Chem.* **294,** 9377–9389 CrossRef Medline
4. Paez, J. G., Jänne, P. A., Lee, J. C., Tracy, S., Greulich, H., Gabriel, S., Herman, P., Kaye, F. J., Lindeman, N., Boggon, T. J., Naoki, K., Sasaki, H., Fujii, Y., Eck, M. J., Sellers, W. R., Johnson, B. E., and Meyerson, M. (2004) EGFR Mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* **304,** 1497–1500 CrossRef Medline
5. Lynch, T. J., Bell, D. W., Sordella, R., Gurubhagavatula, S., Okimoto, R. A., Brannigan, B. W., Harris, P. L., Haserlat, S. M., Supko, J. G., Haluska, F. G., Louis, D. N., Christiani, D. C., Settleman, J., and Haber, D. A. (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.* **350,** 2129–2139 CrossRef Medline
6. Jiang, J., Greulich, H., Jänne, P. A., Sellers, W. R., Meyerson, M., and Griffin, J. D. (2005) Epidermal growth factor–independent transformation of Ba/F3 cells with cancer-derived epidermal growth factor receptor mutants induces gefitinib-sensitive cell cycle progression. *Cancer Res.* **65,** 8968–8974 CrossRef Medline
7. Brenan, L., Andreev, A., Cohen, O., Pantel, S., Kamburov, A., Cacchiarelli, D., Persky, N. S., Zhu, C., Bagul, M., Goetz, E. M., Burgin, A. B., Garraway, L. A., Getz, G., Mikkelsen, T. S., Piccioni, F., *et al.* (2016) Phenotypic characterization of a comprehensive set of MAPK1/ERK2 missense mutants. *Cell Rep.* **17,** 1171–1183 CrossRef Medline
8. Kobayashi, S., Boggon, T. J., Dayaram, T., Jänne, P. A., Kocher, O., Meyerson, M., Johnson, B. E., Eck, M. J., Tenen, D. G., and Halmos, B. (2005) EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.* **352,** 786–792 CrossRef Medline
9. Zhang, X., Gureasko, J., Shen, K., Cole, P. A., and Kuriyan, J. (2006) An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor. *Cell* **125,** 1137–1149 CrossRef Medline
10. Jura, N., Endres, N. F., Engel, K., Deindl, S., Das, R., Lamers, M. H., Wemmer, D. E., Zhang, X., and Kuriyan, J. (2009) Mechanism for activation of the EGF receptor catalytic domain by the juxtamembrane segment. *Cell* **137,** 1293–1307 CrossRef Medline