IN THE SPOTLIGHT

Something Old, Something New, Something Borrowed, Something Fused: Novel EGFR Rearrangements in Lung Adenocarcinomas

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Summary: Mutations in EGFR stand as the archetype for somatic alterations that lead to oncogene addiction and that predict for response to targeted therapies. In this issue of Cancer Discovery, Konduri and colleagues report on a pair of novel oncogenic and actionable EGFR fusion events in a series of patients with lung adenocarcinomas, casting new light on this model gene. Cancer Discov. 6(6): 574–5. ©2016 AACR.

See related article by Konduri et al., p. 601 (5).

The shift in classifying non–small cell lung cancers (NSCLC) by their molecular aberrations first and their histologies second, although not yet complete, is a reflection of the growing success of targeted therapeutics in this disease. Where histology once dictated the selection of a matched treatment (i.e., pemetrexed for nonsquamous NSCLCs), it is increasingly used now as a guidepost for molecular testing: as a means to a more precise therapeutic end. This has been driven largely by the rapid pace at which actionable oncogenic events have been identified. A driver can now be found in most patients’ tumors. Over the past five years, there has also been a change in the nature of these newly identified targets as access to more comprehensive molecular testing tools has been brought into clinical use.

Hence the recent identification of a series of gene rearrangements through next-generation DNA and RNA sequencing (NGS) approaches, including those that involve RET, ROS1, NTRK1, and FGFR1/3 (1–4). These events are rare, not exceeding 1% to 2% per rearrangement, although they have been immediately translatable given the availability of existing tyrosine kinase inhibitors (TKI) that either have been FDA-approved or are under investigation in other indications. Commonalities across these rearrangements have been identified, with two features that are generally present. First, each rearrangement contains the intact kinase domain of the relevant proto-oncogene. Second, the rearrangement partners, although varied, appear to be functionally important, often containing coiled coil domains which can facilitate dimerization and/or localization of the fusion protein.

In this issue of Cancer Discovery, Konduri and colleagues (5) report on a pair of novel fusion events involving the archetype for targeted therapeutics in NSCLC—EGFR and two 3′ partners, RAD51 and PURB—in a series of 5 patients with stage IV lung adenocarcinomas. Each of the patients’ tumors was tested in real time with the hope of finding an actionable target using a clinically validated hybrid capture-based NGS platform (FoundationOne). Four patients had tumors harboring an EGFR fusion with a 5′ breakpoint within intron 24 (EGFR–RAD51). The other harbored an EGFR fusion with a breakpoint at exon 25 (EGFR–PURB). Both, consequently, had retention of an intact EGFR kinase domain. The EGFR variants were fused to either exons 4 to 9 of RAD51 or the 3′ untranslated region of PURB. Overall, fusion events involving EGFR exons 23–intron 25 proved to be rare, occurring in about 0.05% of more than 10,000 NSCLC tumors tested.

Preclinical modeling of the EGFR–RAD51 fusion validated its oncogenic potential in vitro. Stable transfection of this variant in Ba/F3 cells led to IL3-independent growth and downstream activation of the PI3K and MAPK pathways to a similar extent as in Ba/F3 cells expressing EGFRL858R. EGFR–RAD51 expression in NR6 cells (an NIH 3T3 variant that lacks endogenous EGFR) was similarly transforming, leading to increased colony formation when compared to parental cells.

The authors confirmed that the first,-second-, and third-generation EGFR TKIs erlotinib, afatinib, and osimertinib could inhibit growth and signaling in EGFR–RAD51-expressing Ba/F3 cells with low nanomolar IC_{50}s that were not significantly different from those seen in EGFRL858R-expressing cells. EGFR–RAD51-expressing cells were, however, more sensitive to growth inhibition by cetuximab than were EGFRL858R-expressing cells. These in vitro data were in keeping with the durable partial responses reported in all 4 patients who were treated with erlotinib in this report (N = 3, EGFR–RAD51; N = 1, EGFR–PURB). The therapeutic potential of this work alone is important for its immediate clinical relevance.

As intriguing, however, are the structure-function questions that the fusion events bring to light. RAD51 is a eukaryotic homologous recombinaise that self-assembles into filaments containing several RAD51 protomers (6). PURB (encoded by PURB) is a member of a family of ssDNA and RNA binding proteins and functions as a transcriptional represor in vascular smooth muscle cells and myofibroblasts (7).
Prior studies have demonstrated that PURβ can also self-associate into homodimeric complexes (7). This raises the possibility that both RAD51 and PURβ could facilitate EGFR dimerization, which would be in keeping with the presumed function of the nonkinase partners in other oncogene rearrangements. To test this hypothesis, the authors performed in silico modeling of EGFR–RAD51 based on existing structural data and showed that asymmetric dimerization of EGFR is at least geometrically possible. Whether dimerization does indeed occur in practice in either event is currently unknown.

It is worth highlighting in light of these observations that EGFR C-terminal domain (CTD; spanning exons 25–28) deletions have been previously identified in glioblastomas and lung adenocarcinomas (8). This domain is effectively deleted in the EGFR-RADS1 and EGFR-PURB fusion events. CTD deletions vary in extent, from truncations of the entire CTD to partial deletions of it. At least three of these variants—an exon 27 deletion, exon 25–27 deletion, and exon 25–28 deletion—are transforming, as previously shown by Cho and colleagues (8). In addition, these CTD variants sensitize cells to growth inhibition with erlotinib or cetuximab. Intriguingly, Cho and colleagues had also identified a novel CTD variant in an EGFR wild-type infected Ba/F3 clone that unexpectedly exhibited IL3 independent growth. This variant was marked by an intragenic deletion of residues encoding amino acids 1,010 to 1,152 (referred to as CT Del1). Reexpression of the CT Del1 variant into parental Ba/F3 cells and NIH-3T3 cells was transforming. An orthotopic LN443 glioblastoma xenograft model stably expressing CT Del1 also generated brain tumors whose growth could be inhibited by cetuximab.

The CT Del1 variant is intriguing because of its focal nature. The deletion includes tyrosine 1045, which serves as the main binding site for the CBL ubiquitin ligase that targets EGFR for degradation. Consistent with deletion of Y1045, Konduri and colleagues found that the EGFR–RAD51 fusion exhibited decreased EGFR turnover. As the authors noted, the CTD also contains a number of autophosphorylated and transphosphorylated docking sites for adapters that can potentiate EGFR signaling, though these are not necessary for activation of the receptor (9). Taken together with other experimental data, the work by Konduri and colleagues suggests that the EGFR–RAD51/PURB fusions might increase EGFR activity through two mechanisms: facilitation of asymmetric dimerization and/or impairment of receptor turnover. Among the latter is the presumptive mechanism of action for the recently identified oncogenic exon 14 deletion in the MET proto-oncogene, which eliminates the Y1003 CBL-binding site and leads to receptor stabilization, providing a proof-of-principle for this concept (10).

In summary, Konduri and colleagues have identified novel, actionable, and rare EGFR fusions whose existence adds to the growing body of literature that highlights the relevance of somatic alterations that occur outside of the kinase domain. This genotype-to-phenotype correlation was made possible by the increasingly common use of NGS in real-time patient care, serving as a powerful example of personalized medicine in this disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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