IN THE SPOTLIGHT

MTOR Mutations in the Crosshairs of Targeted Therapy

Paul A. Rejto and Robert T. Abraham

Summary: The identification of genetic lesions that affect tumor sensitivity to targeted therapies is a major objective of precision medicine. Two reports in this issue combine tumor genome analyses with functional characterization to uncover activating mutations in *MTOR* that confer sensitivity to a clinically used mTOR inhibitor. *Cancer Discov; 4(5); 513–5. ©2014 AACR.*

See related article by Wagle et al., p. 546 (2).
See related article by Grabiner et al., p. 554 (3).

The principal goal of precision medicine is to define patient populations that are most likely to be responsive or resistant to targeted therapies. Stunning advances in technologies related to cancer genome characterization have made it possible to unveil both common and relatively rare allelic variants that yield protein products with the potential to significantly affect responses to targeted therapies. The cancer genome landscape is marked by relatively few "mountains" that represent the most commonly mutated genes (e.g., *KRAS*), and vast numbers of "hills" that represent low-frequency but potentially actionable genetic alterations. Individually, a low-frequency lesion affecting a druggable gene product marks a relatively small population of patients with cancer for targeted therapy. If the same protooncogene can achieve its oncogenic potential through multiple, low-frequency alterations, then the summation of all possible oncogenic variants may represent a substantial population of patients who will benefit from targeted therapy. This scenario has stimulated increasing interest in the identification of rare variants that underpin the malignant phenotype.

The PI3K–AKT–mTOR pathway stimulates metabolic events required for cell growth and proliferation, and has attracted intense interest from both the academic and industrial sectors due in part to its widespread hyperactivation in human cancers (1). Actionable drug targets in this pathway fueled considerable investment in the discovery and development of pharmacologic inhibitors, many of which target, more or less selectively, phosphoinositide 3-kinase (PI3K) isoforms and/or mTOR. Despite the wealth of genetic evidence supporting PI3K pathway dysregulation as a major driver in human cancer, the identification of patients with cancer who are most likely to respond to these inhibitors remains a significant challenge. Perhaps many of the truly meaningful predictive biomarkers reside in low-frequency mutations that hyperactivate signaling through the PI3K pathway.

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doi: 10.1158/2159-8290.CD-14-0332

Gene sets that exhibit unusually high rates of somatic mutations are enriched in oncogenic drivers, particularly when these mutations are in regionally localized clusters. The demonstration that a particular genetic lesion alters the function of the protein product in a direction consistent with oncogenesis greatly increases confidence in its assignment as a disease driver, rather than an inconsequential passenger. The mirror image of this genotype-to-phenotype approach involves the identification of patients who exhibit extraordinarily positive responses to targeted therapies in clinical trials, followed by analyses of tumor genotypes, with the goal of identifying genetic alterations that underlie the exceptional drug responses.

Two articles in this issue of *Cancer Discovery* highlight the utility of the genotype-to-phenotype and extreme-responder approaches when combined with functional evaluations (2, 3). The gene of central interest in both articles is *MTOR*, a major drug target in the PI3K signaling cascade. Before these new reports, only a few missense *MTOR* mutations leading to hyperactivation of mTOR-dependent signaling had been discovered, and the impact of these activating mutations on mTOR-targeted therapies was undefined (4, 5). Grabiner and colleagues (3) performed the first comprehensive survey of mTOR pathway–associated genes, searching cancer cell line and tumor genomic databases for missense (amino acid sequence–altering) mutations that might alter mTOR signaling functions in human cancer. This search uncovered somatic mutations in virtually every component of the PI3K–mTOR pathway. The unanticipated result was that the *MTOR* gene itself was the single richest source of missense mutations among all pathway components surveyed. The analyses by Grabiner and colleagues (3) netted a treasure trove of mTOR-activating mutations (33 in total) that could be subgrouped into six clusters, suggesting that these regionally localized mutations were positively selected during tumorigenesis. Furthermore, the clusters were located in the carboxyl-terminal half of the mTOR polypeptide, which contains several functionally important domains, including the protein kinase domain as well as the flanking FKBP12-rapamycin binding (FRB), FAT, and FATC domains (Fig. 1). The impact of these mutations on mTOR activity was assessed by transfection into a human cell line host, followed by examination of the phosphorylation of downstream substrates of the two mTOR-containing complexes, mTORC1 and mTORC2. The authors explored the effects of the mutations on the associations of mTOR with other mTORC1 and

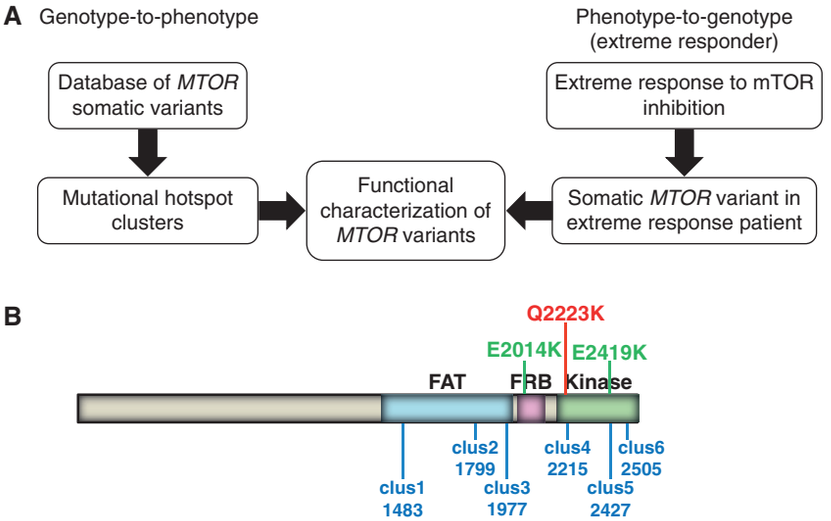

Figure 1. **A,** a flowchart of the genotype-to-phenotype and phenotype-to-genotype (extreme responder) approaches leading to candidate predictive biomarkers of response to mTOR inhibitors. **B,** schematic diagram of the 2549 amino acid, human mTOR protein highlighting the FAT, FRB, and kinase domains. The locations of the peaks of the six activating mutation clusters identified by Grabiner et al. (3) are shown in blue, the two activating *MTOR* mutations identified by Wagle et al. (2) in the patient with urothelial cancer are depicted in green, and the *MTOR* mutation identified in an extreme responder with renal cancer (8) is depicted in red.

mTORC2 partner proteins, and found that all of the tested mTOR mutants coimmunoprecipitated reduced amounts of DEPTOR, a stoichiometric inhibitor of mTORC1 and mTORC2 signaling functions (6). Furthermore, Grabiner and colleagues (3) found that several of the mTOR mutants rendered mTORC1 activity abnormally resistant to nutrient deprivation, which might confer a distinct survival advantage to tumor cells residing in metabolically stressful microenvironments (7). Finally, the authors reported that the identified mutations did not confer resistance to rapamycin and other mTOR inhibitors; indeed, cancer cell lines that naturally harbored mutationally activated forms of mTOR were *hypersensitive* to the growth-inhibitory effects of rapamycin *in vitro*.

The second report in this issue of *Cancer Discovery* nicely complements the above findings by demonstrating that mutational activation of mTOR can in fact mark a human tumor for increased therapeutic responsiveness to an mTOR inhibitor (2). Wagle and colleagues (2) enrolled a small cohort of advanced cancer patients with different histologic tumor types in a clinical trial involving combination therapy with everolimus, an mTORC1 inhibitor, plus pazopanib, a multitargeted receptor tyrosine kinase inhibitor with antiangiogenic activity. One patient with urothelial carcinoma experienced a remarkable, complete radiographic response that lasted nearly 14 weeks. Whole-exome sequencing of this patient's tumor uncovered two mutations in *MTOR*, one located in the kinase domain and another in the FRB domain (Fig. 1). Using a cell transfection approach similar to that used by Grabiner and colleagues (3), the authors showed that each mutation individually activated mTOR signaling in the host cells, and that the two mutations together exerted an additive stimulatory effect on mTOR-dependent signaling (2). Structural modeling of the doubly mutated mTOR variant indicated no detectable alteration in the protein kinase domain, suggesting that, similar to the findings of Grabiner and colleagues (3), these mutations activated mTOR by disrupting the negative regulatory influence of DEPTOR or another, unknown binding partner.

These two new reports highlight both the opportunities and the challenges facing both oncologists and patients as cancer genome characterization becomes embedded into the disease-management paradigm. Gene sequencing alone provides clues about potential oncogenic alterations, with further supporting evidence coming from functional assessments such as those performed by Grabiner and colleagues (3). However, this approach falls short of proving that established tumors bearing the identified mutations are actually driven by the mutated mTORs, or the corollary assumption that these tumors will be clinically responsive to mTOR-targeted therapies. This shortcoming was partially addressed by an independent team of investigators, who identified a patient with renal cancer whose tumor displayed an extreme response to everolimus, and was subsequently shown to bear a *MTOR* mutation identical to one of the alterations identified by Grabiner and colleagues (3, 8). Interestingly, constitutive hyperactivation of mTORC1 is expected to provoke negative feedback inhibition of PI3K–AKT signaling, potentially leading to tumor growth suppression (9). This feedback loop may be disrupted in cancer cells in which mutationally activated mTOR serves as a *bona fide* oncogenic driver.

The extreme-responder approach exemplified by Wagle and colleagues (2) has also been termed an "N of 1" experiment, which accurately sums up both the excitement and the challenges generated by such a study (10). The demonstrated linkage between a dramatic clinical response phenotype and a specifically altered genotype underscores the promise of precision medicine. However, the N of 1 nature of extreme-responder studies carries some practical limitations. By definition, an "N of 1" study lacks replication; hence, one cannot rule out the possibility that the extreme responder is a statistical outlier and that the positive outcome will not be reproduced in a larger population of appropriately selected patients. Other variables may also complicate extrapolation of N of 1 outcomes to additional patients. For example, Wagle and colleagues (2) acknowledged that their patients received combination therapy with everolimus plus pazopanib, and that a potential contribution of pazopanib to the extraordinary response in this patient with urothelial cancer could not be excluded.

The studies described above shed additional light on a fast-approaching, revolutionary change in the design of oncology

clinical trials. Like the *MTOR* mutations described in these two reports, many of the drug sensitivity–conferring mutations in human cancers will be expressed at low frequencies (11), indicating that many more patients must be screened to enroll sufficient numbers to draw statistically valid conclusions about drug efficacy. Contextual differences that promote drug resistance in tumors bearing an otherwise sensitive genotype will also need to be assessed in parallel with the molecular target(s) of the test drug. Intratumoral heterogeneity is a looming, additional challenge that demands rationally designed combinations of targeted therapies (5). Although these and other considerations will require significant changes in the current cancer clinical trial paradigm, the goal of making extreme clinical responses far more commonplace is well worth the effort.

Disclosure of Potential Conflicts of Interest

P.A. Rejto has ownership interest (including patents) in Pfizer. R.T. Abraham is employed as Chief Scientific Officer–Oncology at Pfizer and has ownership interest (including patents) in the same.

Published online May 2, 2014.

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Cancer Discovery 2014;4:513-515.