IN THE SPOTLIGHT

mTOR Inhibition, the Second Generation: ATP-Competitive mTOR Inhibitor Initiates Unexpected Receptor Tyrosine Kinase–Driven Feedback Loop

Megan Keniry[1] *and Ramon Parsons*[1,2]

Summary: mTOR inhibition with the ATP-competitive kinase inhibitor AZD8055 induces receptor tyrosine kinase–dependent feedback activation of AKT. *Cancer Discovery; 1(3);* 203–4. *©2011 AACR.*

Commentary on Rodrik-Outmezguine et al., p. 248 (1).

In this issue of *Cancer Discovery*, Rodrik-Outmezguine and colleagues (1) discover a surprising biphasic regulation of AKT after inhibition of mTOR kinase complexes TORC1 and TORC2. This novel feedback mechanism was uncovered in numerous cancer cell lines and xenografts that were treated with AZD8055, an ATP-competitive mTOR kinase inhibitor, which potently and selectively targets both mTORC1 and mTORC2 activities. Predictably, AZD8055 treatment led to changes in mTORC1 and mTORC2 outputs, such as the phosphorylation of p70S6K (Thr389), 4EBP1 (Thr37/46, Thr70, and Ser65) and AKT (Ser473; ref. 1). Unexpectedly, AKT kinase activity recovered 8 hours after AZD8055 treatment, as evidenced by an increase in phospho AKT (Thr308) and concomitant phosphorylation of its substrates (1). This heightened AKT output required receptor tyrosine kinase (RTK) activity and highlights a novel feedback mechanism that can bypass the requirement of mTORC2 for AKT activation.

The mTOR serine/threonine kinase resides in multiprotein complexes, mTORC1 and mTORC2, which regulate cellular proliferation, size, survival, and translation (2). mTORC1 is composed of mTOR, RAPTOR, mLST8, PRAS40, and DEPTOR (2). Nutrient availability regulates mTORC1 in its ability to phosphorylate p70S6K and 4EBP1 in order to induce protein synthesis (2). In addition to regulating protein synthesis, the mTORC1 complex attenuates insulin signaling by negatively regulating IRS1 and activating GRB10 (3–5). The mTORC2 complex, in contrast, contains mTOR, RICTOR, mLST8, DEPTOR, SIN1, and PROTOR (2). mTORC2 targets include AKT, PKCα, and SGK1, which regulate cellular growth and survival (2). The central role of mTOR in these growth regulatory events makes it a rational target for cancer therapy.

mTOR inhibitors vary in target selectivity and mechanism. The first-generation mTOR inhibitors rapamycin and its analogues—sirolimus, temsirolimus, everolimus, and deforolimus—employ an allosteric mechanism to block mTORC1 output (6). In contrast, second generation mTOR inhibitors such as AZD8055, Torin1, PP242, and PP30 competitively target the ATP binding site to impede kinase activity of both TORC1 and TORC2 (6). In addition to these drugs, dual ATP-binding kinase inhibitors of mTOR and phosphoinositide 3-kinase (PI3K; NVP-BEZ253 and PI-103) may prove more efficacious by inhibiting oncogenic signaling events to a greater extent (6). In this issue of *Cancer Discovery*, Rodrik-Outmezguine and colleagues (1) show a side-by-side comparison of mTORC inhibition with rapamycin and AZD8055, which revealed surprising changes in mTOR signaling. Rapamycin treatment led to an almost complete loss in the mTORC1 phosphorylation of p70S6K (Thr389) with essentially no impact on the phosphorylation of 4EBP1 (Thr65 and Thr70), whereas phospho AKT (Ser473) was increased. In contrast, AZD8055 treatment led to strong reductions in phospho 70S6K (Thr389), phospho 4EBP1 (Thr37/40, Thr65, and Thr70) and phospho AKT (Ser473; ref. 1). On the basis of these findings, AZD8055 is a better inhibitor of mTORC1 in comparison to rapamycin, consistent with another recent AZD8055 study by Chresta et al. (7). *In vivo* studies indicate that AZD8055 can inhibit tumor growth and can even lead to tumor regression when combined with lapatinib. Therefore, AZD8055 shows promise as a therapeutic agent, but harnessing the full potential will require a greater understanding of the web of feedback loops that intertwine PI3K, mTOR, and RTKs.

An astonishing result from Rodrik-Outmezguine and colleagues (1) is the rebound in AKT activity in the absence of mTORC2. Scores of studies use levels of phopho AKT (Ser473) as a barometer of AKT activity. Yet, in this study AKT output is high after 8 hours of AZD8055 treatment, even though phospho AKT (Ser473) is low. The logic follows that AKT output and phospho AKT (Ser 473) may be discordant in other instances. Conceivably, phospho AKT (Thr308), which is modified on a key T-loop residue, is a more reliable indicator of AKT activity. AKT phosphorylation on Thr308 is essentially required for kinase activity (8). Phosphorylation on Thr308 increases AKT kinase activity by at least 100-fold, whereas phosphorylation on Ser473 mediates stabilization of the active conformation of the kinase, increasing activity by roughly another 10-fold (9). The gain in AKT output in the absence of Ser473 phosphorylation suggests that the active conformation of AKT may be stabilized in another manner, perhaps by interacting with the hydrophobic motif of another protein.

Authors' Affiliations: [1]Institute for Cancer Genetics and Herbert Irving Comprehensive Cancer Center, Columbia University; [2]Departments of Pathology and Cell Biology, and of Medicine, Columbia University Medical Center, New York, New York

Corresponding Author: Ramon Parsons, Institute for Cancer Genetics and Herbert Irving Comprehensive Cancer Center, Columbia University, 1130 St. Nicholas Avenue, New York, NY 10032. Phone: 212-851-5278; Fax: 212-851-5267; E-mail: rep15@columbia.edu

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The recovery of phospho AKT (Thr308) with AZD8055 treatment is dependent on RTK activation. It remains to be determined exactly how these RTKs are activated upon mTORC1/2 inhibition. On the basis of previous studies, IRS1 should be more stable upon mTOR inhibition, which would enhance RTK output (3). However, the observed increase in RTK activities [epidermal growth factor receptor, HER2, HER3, HER4, IGF1R, insulin receptor (IR), and FGF1–3 families] after AZD8055 treatment suggests a more potent and broadly applicable connection between mTOR and RTK output.

Feedback mechanisms are becoming a recurring theme in oncogenic signaling. Table 1 summarizes numerous feedback mechanisms that impinge on PI3K, mTOR, and RTK output. In addition to the RTK induction mediated by mTOR inhibition, targeting PI3K, AKT, and mTORC1 also induces feedback responses. Treatment with the PI3K inhibitor LY294002 can stabilize the associations between the p85 regulatory subunit of PI3K with the IR as well as p85 with tyrosine-phosphorylated IRS1 and IRS2, which can lead to activation of active PI3K complexes (10, 11). In addition, exogenous PTEN is capable of eliciting a transcriptional feedback induction of *IR* and *IRS2* expression (11). Recently, Chandarlapaty and colleagues (12) found that AKT inhibition induced FOXO-dependent induction of RTK signaling, which impeded cell death. In addition to this, Carracedo and colleagues (13) observed that rapamycin treatment induced ERK signaling. Taken together, these studies reveal a complex tapestry of interconnections between PI3K, mTOR, and RTKs. Hence, to successfully target PI3K, mTOR, and RTK outputs, one may need to employ combination therapies to hinder feedback mechanisms.

Combination therapies that target mTOR, PI3K, and RTK activities show tremendous potential as areas that could advance therapeutic options for cancer. Chandarlapaty and colleagues (12) showed that the combination of AKT inhibition with gefitinib (Iressa) or lapatinib leads to an increase in tumor regression in xenograft models. Additional studies have revealed that combined PI3K and ERK inhibition is better able to target BAD and 4EBP1 (14, 15). In studies by Rodrik-Outmezguine and colleagues (1), an increase in tumor regression was observed with combined AZD8055 and lapatinib in a xenograft model. The ability of these combinations to perform in the clinic will be a matter of intense investigation for years to come. Future endeavors will need to integrate advances in inhibitor development with strategies for addressing novel feedback mechanisms as they emerge.

Table 1. PI3K/mTOR/RTK feedback mechanisms

Feedback mechanism	Author (reference)
PI3K inhibition with LY294002 stabilizes the interactions between p85 and IR as well as p85 with IRS1.	Rameh et al. (10)
Exogenous PTEN induces the expression of *IR* and *IRS2*.	Simpson et al. (11)
mTOR/p70S6K inhibit IRS1.	Shah and Hunter (3)
mTORC1 phosphorylates GRB10 to inhibit insulin signaling.	Yu et al. (4), Hsu et al. (5)
AKT inhibition activates RTK expression through FOXO.	Chandarlapaty et al. (12)
Rapamycin treatment induces ERK activity.	Carracedo et al. (13)
Selective ATP-competitive mTOR inhibitors induce RTK activation and expression.	Rodrik-Outmezguine et al. (1)

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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