TORgeting oncogene addiction for cancer therapy

The PI3K-Akt-mTOR growth-regulating pathway is conserved from mammals to flies and hyperactivated in many cancers. Accordingly, rapamycin analogs, which are inhibitors of mTOR-Raptor signaling, have recently garnered much interest as potential therapeutic agents against cancer. However, due to the heterogeneity of tumors, prior knowledge of the genetic and biochemical background of cancer cells will be required for effective targeted therapy. Thus, the identification of biological markers against activated oncogenic pathways is needed. In the January issue of *Nature Medicine*, Thomas et al. identify the loss of *VHL* tumor suppressor gene as a potential determining factor in tumor sensitivity to rapamycin.

The phosphatidylinositol 3-kinase (PI3K) pathway is an evolutionarily conserved lipid kinase that initiates a series of growth factor-activated signals that provide the cell with adequate biosynthesis to meet biological demand for basic processes such as cellular growth, proliferation, survival, and bioenergetics. Accordingly, it is not surprising that mutations that inappropriately activate this pathway are associated with various malignancies, including cancers of the colon, breast, liver, brain, stomach, lung, and ovary. *PTEN*, a negative regulator of this pathway, is a tumor suppressor whose function is lost through mutation, inactivation, or silencing in a multitude of cancers. For example, *PTEN* mutations have been identified in 45% of carcinomas of the endometrium and greater than 20% of glioblastomas (extensive list reviewed in Ali et al., 1999). Recently, activating mutations in PI3Kα have also been identified in many human malignancies (Bader et al., 2005). Thus, through a variety of mechanisms, a high percentage of human cancers possess activated PI3K signaling. Downstream effectors of the PI3K pathway include Akt and the mTOR complex 1 (TORC1), which constitutes mTOR, Raptor, and mLst8 (GβL). Akt is a well-known cell survival kinase (Bader et al., 2005) that also phosphorylates and antagonizes the tumor suppressor TSC2, a GAP that inhibits the G protein Rheb. Rheb is a positive regulator of TORC1 activation that links Akt to cell growth control. Thus, Akt integrates PI3K signaling with the TORC1 nutrient and energy-sensing pathway (Fingar and Blenis, 2004). It is currently believed that TORC1 mediates its progrowth effects through the activation of S6 kinase 1 (S6K1) and suppression of 4E-BP1, an inhibitor of cap-dependent translation (Fingar and Blenis, 2004). These observations all point to mTOR-Raptor as a critical target in cancer therapy, and indeed, rapamycin analogs (CCI-779, RAD001, AP23576) are currently undergoing clinical trials for the treatment of renal cell carcinomas, metastatic breast cancers, lymphomas, and other malignancies.

Despite the essential role that mTOR plays in meeting the biosynthetic demand for tumor propagation, early clinical results suggest that patient responsiveness to mTOR inhibition is variable. For instance, a phase II trial against metastatic melanoma demonstrated that CCI-779 alone was insufficient to provide tumor cytostasis using concentrations and a dosing schedule supported by phase I and pharmacodynamic studies

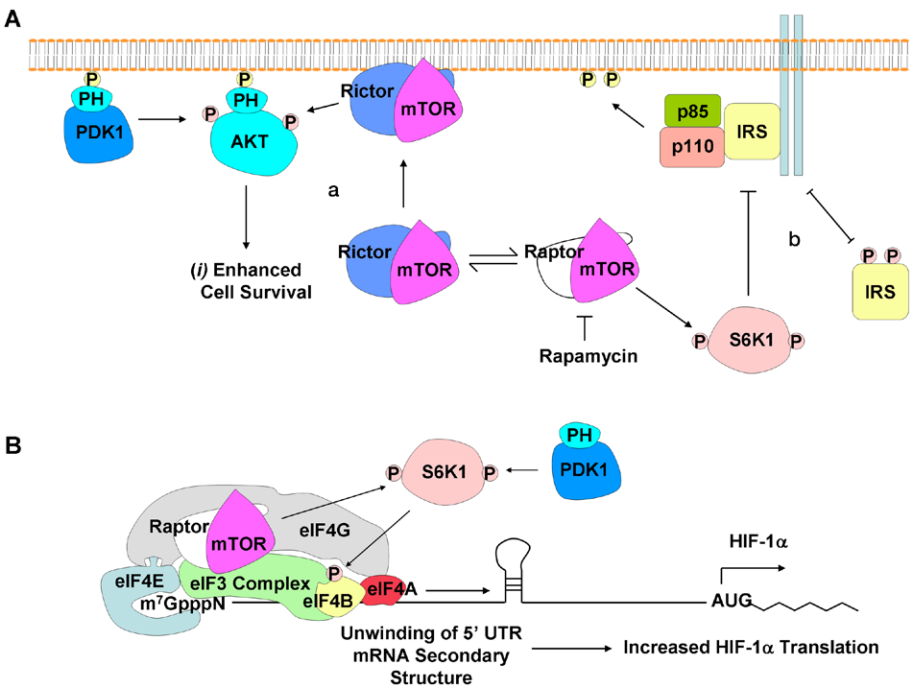

Figure 1. Rapamycin-mediated regulation of HIF-1α translation and potential implications of long-term rapamycin treatment

Aa: mTOR exists in two mutually exclusive complexes with Raptor or Rictor. The rapamycin-insensitive mTOR-Rictor complex phosphorylates Ser473 on Akt, a critical regulatory site that is required for activation. Long-term rapamycin treatment shifts the equilibrium of these two complexes toward mTOR-Rictor (Sarbassov et al., 2005), leading to increased overall Akt activity and cellular survival.

Ab: Rapamycin treatment inhibits a S6K1-mediated negative feedback loop that normally suppresses Akt activation. In many cancers, the insulin receptor substrate-1 (IRS-1) is recruited to engaged receptors like the IGF-1 receptor and mediates PI3K recruitment to the membrane to generate D-3 phosphoinositides (PIP3) (yellow phosphates), leading to Akt activation. S6Ks phosphorylate IRS-1, promoting its dissociation from the receptor, which decreases PIP3 production and attenuates Akt activation. Thus, in some cancers rapamycin can result in increased Akt activity and tumor cell survival by one or both mechanisms.

B: Upon growth factor activation, S6K1 is phosphorylated by mTOR-Raptor at its hydrophobic motif site and released from the eIF3-40S preinitiation complex (PIC). The activated S6K1 then phosphorylates eIF4B (Ser/Thr phosphates in pink), which associates with the PIC to function as a cofactor for the RNA helicase eIF4A (see Holz et al., 2005). This presumably could augment the translation of the HIF-1α mRNA by alleviating secondary mRNA structural constraints within its 5'UTR and hence rendering the process rapamycin sensitive.

(Margolin et al., 2005). Therefore, understanding the mechanism for rapamycin sensitivity and garnering appropriate biological markers for the mTOR pathway are crucial for predicting possible responsiveness to the drug.

In an effort to understand rapamycin sensitivity and to identify biomarkers for potentially responsive patients, Charles Sawyers and colleagues implemented the idea of "pathway or oncogene addiction" in tumors and identified the loss of the tumor suppressor Von Hippel-Lindau (*VHL*) gene as a determining factor in sensitivity to CCI-779 treatment (Thomas et al., 2006). The *VHL* gene encodes an E3 ligase that is responsible for ubiquitination and turnover of the α subunit of the heterodimeric HIF family of transcriptional factors, which promote expression of factors involved in angiogenesis and glycolysis (Kaelin, 2005). The authors used isogenic pairs of renal cell carcinomas differing only in the expression of *VHL* via stable shRNA-mediated knockdown. They first showed that cells expressing *VHL*-specific shRNA exhibited increased HIF-1α/β, GLUT-1, and CA-IX expression, and cellular proliferation presumably through increased HIF-1 stability and transcription. Surprisingly, cells with *VHL* knockdowns, but not control cells, exhibited a profound proliferation defect both in culture and in xenograft tumor experiments when treated with CCI-779. This correlated with a loss of HIF-1α expression upon mTOR inhibition. The effect of CCI-779 on HIF-1α was observed in *VHL* knockdown cells under both normoxic and hypoxic conditions, suggesting that mTOR signaling is absolutely necessary for expression of HIF-1α irrespective of environmental conditions.

The authors then provided mechanistic evidence that mTOR regulates the translation, but surprisingly not the turnover of HIF-1α. More specifically, the effect mediated by mTOR required the 5′UTR structure of HIF-1α mRNA. CCI-779 suppressed translation of the wild-type HIF-1α message but not the translation of HIF-1/2α ubiquitination mutants lacking the 5′UTR region. This effect was also observed in xenografts, as these tumors were completely recalcitrant to CCI-779 treatment when compared to the control expressing HIF-1α mRNA with a wild-type 5′UTR. The implications from these data are that the loss of *VHL* and the subsequent upregulation of HIF-1α rendered these tumors "addicted" to the proliferative advantage provided by the oncogenic activity of this transcription factor. Cancer cells lacking VHL are then unable to proliferate when suddenly deprived of HIF-1α by CCI-779-mediated translational repression. Importantly, this effect was also observed in other renal cell carcinomas with naturally occurring *VHL* mutations.

In an effort to identify a noninvasive method for predicting the efficacy of CCI-779 treatment, the authors took advantage of previous observations that prostate cells transformed with Akt showed elevated glycolysis likely through a HIF-1α- and mTOR-dependent upregulation of various glycolytic enzymes and the glucose transporter GLUT-1 (Majumder et al., 2004). Accordingly, the authors used fluorodeoxyglucose (FDG), a glucose analog, as a tracer for positron emission tomography (PET) analysis. The authors showed that, in xenografts, the CCI-779-treated *VHL* knockdown cells exhibited a dramatically decreased ability to take up FDG. Importantly, this effect was not seen when mice bearing the *VHL* shRNA-induced tumors were treated with Paclitaxel, suggesting that this effect is specific to mTOR inhibition.

The authors hypothesized that the mechanism behind the requirement of mTOR activation for HIF-1α translation is likely through the phosphorylation of the 40S ribosomal protein S6 by the mTOR target S6K1, which has been proposed to regulate selective translation of 5′-terminal oligopolypyrimidine (TOP)-containing mRNAs, including the HIF-1α message (Thomas et al., 2006). However, recent evidence suggests that neither S6K1 and S6K2 activities nor phosphorylation of S6 is necessary for translation of 5′ TOP-containing mRNAs (Fingar and Blenis, 2004; Ruvinsky et al., 2005). Therefore, it will be interesting to determine if in fact S6K1 and S6K2 are necessary for the translation of HIF-1α. Moreover, mRNAs that possess long and structured 5′UTRs are sensitive to mTOR inhibition via the helicase activity of the eIF4F complex (Fingar and Blenis, 2004), implying that even if S6K1 and S6K2 are required for translation of the HIF-1α mRNA, this effect may not be due to the 5′ TOP structure. Accordingly, S6K1-mediated phosphorylation of eIF4B, an essential cofactor for the eIF4F helicase eIF4A, was shown to be required for eIF4B recruitment to the 5′ cap complex by interacting with the eIF3:40S preinitiation complex (Holz et al., 2005; Figure 1B). Thus, a 5′ TOP-independent mechanism remains a possibility, as the authors' 5′UTR constructs were not based on mutations but were truncations.

The importance of mTOR in cancer cell biology and clinical therapeutics are starting to emerge as the implications of mTOR in cell growth, proliferation, and bioenergetic metabolism are revealed. As in all biomedical fields, translating basic cancer biology into the clinic is absolutely critical, and the work by Thomas et al. effectively amalgamates the biochemical importance of mTOR into a potential clinical target. However, the focus of this study is on HIF-1α-mediated addiction without chronic activation of PI3K signaling as is observed in many cancers. This leads to the question of whether rapamycin will be effective in all cancers or if successful treatment will depend on the extent of PI3K/mTOR activation. Indeed, recent observations are beginning to reveal that treatment with rapamycin should be conducted with caution. For instance, a rapamycin-insensitive mTOR complex referred to as TORC2 (mTOR-Rictor) exists. TORC2 phosphorylates and activates Akt. Importantly, long-term rapamycin treatment can dramatically alter the dynamic equilibrium between these complexes toward TORC2 (Sarbassov et al., 2005). In addition, S6K1 provides an inhibitory feedback loop that can suppress Akt activity. Thus, rapamycin treatment may result in enhanced Akt activation and enhanced tumor growth and survival (Figure 1A). Along these lines, *RNAi*-mediated knockdown of mTOR, but not rapamycin treatment, can potentiate Taxol-mediated cell death in HeLa cells (MacKeigan et al., 2005 and unpublished data), suggesting that a catalytic inhibitor of mTOR may be a more effective antagonist of cancer cell proliferation and survival. Rapamycin may be more useful in combination with other therapies, as reports from several groups suggest that rapamycin enhances the effects of other anti-cancer agents. For instance, rapamycin augments Gleevec-mediated proliferation inhibition of Bcr-Abl-expressing cells in vitro and prolongs the survival of mice with bone marrow transduced with Bcr-Abl (Kharas and Fruman, 2005). In some cancer cells, potentiating effects on apoptosis have been observed when traditional DNA-damaging chemotherapeutics were combined with rapamycin (Hennessy et al., 2005). Thus, the work by Sawyers and coworkers, and

studies combining rapamycin with other specific pathway inhibitors and/or traditional chemotherapeutics, may ultimately provide the necessary arsenal to effectively and successfully combat cancer. Clearly, the success of this battle will also rely heavily on the availability of biomarkers that reveal pathway signaling status in the malignant cells.

Andrew Y. Choo[1,2] and John Blenis[1,*]

[1]Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115
[2]Program in Biological and Biomedical Sciences (BBS), Harvard Medical School, Boston, Massachusetts 02115
*E-mail: john_blenis@hms.harvard.edu

Selected reading

Ali, I.U., Schriml, L.M., and Dean, M. (1999). J. Natl. Cancer Inst. *17*, 1922–1932.

Bader, A.G., Kang, S., Zhao, L., and Vogt, P.K. (2005). Nat. Rev. Cancer *5*, 921–929.

Fingar, D.C., and Blenis, J. (2004). Oncogene *23*, 3151–3171.

Hennessy, B.T., Smith, D.L., Ram, P.T., Lu, Y., and Mills, G.N. (2005). Nat. Rev. Drug Discov. *4*, 988–1004.

Holz, M.K., Ballif, B.A., Gygi, S.P., and Blenis, J. (2005). Cell *123*, 569–580.

Kaelin, W.G. (2005). Annu. Rev. Biochem. *74*, 115–128.

Kharas, M.G., and Fruman, D.A. (2005). Cancer Res. *65*, 2047–2053.

MacKeigan, J.P., Murphy, L.O., and Blenis, J. (2005). Nat. Cell Biol. *7*, 591–600.

Majumder, P.K., Febbo, P.G., Bikoff, R., Berger, R., Xue, Q., McMahon, L.M., Manola, J., Brugarolas, J., McDonnell, T.J., Golub, T.R., et al. (2004). Nat. Med. *10*, 594–601.

Margolin, K., Longmate, J., Baratta, T., Synold, T., Christensen, S., Weber, J., Gajewski, T., Quirt, I., and Doroshow, J.H. (2005). Cancer *104*, 1045–1048.

Ruvinsky, I., Sharon, N., Lerer, T., Cohen, H., Stolovich-Rain, M., Nir, T., Dor, Y., Zisman, P., and Meyuhas, O. (2005). Genes Dev. *19*, 2199–2211.

Sarbassov, D.D., Guertin, D.A., Ali, A.M., and Sabatini, D.M. (2005). Science *307*, 1098–1101.

Thomas, G.V., Tran, C., Mellinghoff, I.K., Welsbie, D.S., Chan, E., Fueger, B., Czernin, J., and Sawyer, C.L. (2006). Nat. Med. *12*, 122–127.

DOI 10.1016/j.ccr.2006.01.021