mTOR and cancer: insights into a complex relationship

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Abstract | mTOR (mammalian target of rapamycin) has come a long way since its humble beginnings as a kinase of unknown function. As part of the mTORC1 and mTORC2 complexes mTOR has key roles in several pathways that are involved in human cancer, stimulating interest in mTOR inhibitors and placing it on the radar of the pharmaceutical industry. Here, I discuss the rationale for the use of drugs that target mTOR, the unexpectedly complex mechanism of action of existing mTOR inhibitors and the potential benefits of developing drugs that function through different mechanisms. The purpose is not to cover all aspects of mTOR history and signalling, but rather to foster discussion by presenting some occasionally provocative ideas.

In response to growth factors and nutrients mTORC1 (mammalian target of rapamycin complex 1) regulates cell growth by modulating many processes, including protein synthesis, ribosome biogenesis and autophagy (reviewed in REF. 1). mTORC1 is a heterotrimeric protein kinase that consists of the mTOR catalytic subunit and two associated proteins, raptor (regulatory-associated protein of mTOR) and mLST8 (also known as GBL) (BOX 1). The molecular mechanisms that regulate mTORC1 kinase activity are still poorly understood, but it is increasingly clear that many if not most cancer-promoting lesions activate the mTORC1 pathway (FIG. 1). Most dramatically, the TSC1 (tuberous sclerosis 1, also known as hamartin)–TSC2 (also known as tuberin) tumour suppressor complex — the inactivation of which causes the tumour-prone syndrome tuberous sclerosis complex (TSC) and the related disease lymphangioleiomyomatosis (LAM) — has emerged as a key negative regulator of mTORC1 (REFS 2,3). The TSC1–TSC2 heterodimer is a GTPase-activating protein for Rheb (Ras homologue enriched in brain)4–6, a GTP-binding protein that activates mTORC1, most probably by binding to it7. TSC1–TSC2 and Rheb also have important roles in the activation of mTORC1 that occurs when cells lose the PTEN (phosphatase and tensin homologue), NFI (neurofibromatosis 1), LKB1 (also known as serine–threonine kinase 11) or p53 tumour suppressors10–13. In all cases, inactivation of the tumour suppressor triggers a pathway that eventually leads to inhibition of TSC1–TSC2. For example, the loss of PTEN activates Akt (also known as protein kinase B), which then directly phosphorylates and inhibits TSC1–TSC2, whereas the loss of LKB1 suppresses AMPK (AMP-activated protein kinase)14,15, which normally mediates an activating phosphorylation of TSC1–TSC2 (REF. 18).

The mTORC1 pathway regulates growth through downstream effectors, such as the regulators of translation 4EBP1 (eukaryotic translation initiation factor 4E binding protein 1) and S6K1 (ribosomal S6 kinase 1) (reviewed in REF. 19). In addition to its role in promoting protein synthesis, S6K1 represses the phosphatidylinositol 3-kinase (PI3K)–Akt pathway by inhibiting IRS1 (insulin receptor substrate 1) and IRS2 expression20–24. Therefore, an active mTORC1 pathway can suppress PI3K–Akt signalling, helping to explain the non-aggressive nature of the tumours that are found in TSC25,26. The opposite is also true: inhibition of mTORC1 activates PI3K–Akt signalling and, as described below, the activation of PI3K–Akt that is caused by mTORC1 inhibitors might significantly diminish the anti-tumour activity of such molecules.

mTORC2 also contains mTOR and mLST8 but, instead of raptor, it contains two proteins, rictor (rapamycin-insensitive companion of mTOR) and mSin1 (also known as mitogen-activated-protein-kinase-associated protein 1), that are not part of mTORC1 (BOX 1). This second mTOR-containing complex is less understood than mTORC1 but recent work indicates that it should be considered part of the PI3K–Akt pathway as it directly phosphorylates Akt27,28 on one of the two sites that are necessary for Akt activation in response to growth-factor signalling (FIG. 1). This finding makes mTORC2 a key part of the pathway that activates Akt and, like PDK1 (3-phosphoinositide-dependent protein kinase 1) and PI3K, a potential drug target for cancers in which there is Akt deregulation. The Akt-activating function of mTORC2 sets up the intriguing situation in which mTOR, as part of two distinct complexes, is potentially both ‘upstream’ and ‘downstream’ of itself. mTORC2 has other functions besides activating Akt, such as regulating the cytoskeleton29,30, but the implications for cancer of these roles are still unknown.

What does rapamycin do to the mTORCs?
mTOR was discovered in the early 1990s in studies into the mechanism of action of rapamycin (also known as sirolimus), which is a macrolide that was originally found as an antifungal agent and was later recognized as having immunosuppressive and antinecancer properties. Even today, exactly how rapamycin perturbs mTOR function is not completely understood. The complex of rapamycin with its intracellular receptor FKBP12 binds directly to mTORC1 and, at least in vitro, suppresses mTORC1-mediated phosphorylation of the substrates S6K1 and 4EBP1. Rapamycin also weakens the interaction between mTOR and raptor31, which is a component of mTORC1 that can recruit substrates to the mTOR kinase domain32–34. It is not known if mTORC1 has...
functions that depend on its kinase activity but are not sensitive to rapamycin, so it is still unclear if a molecule that directly inhibited the mTORC1 kinase domain would have different biological effects to those of rapamycin. Analogues of rapamycin, such as CCI-779 (also known as temsirolimus; Wyeth), RAD001 (also known as everolimus; Novartis) and AP23573 (Ariad Pharmaceuticals), are likely to be the first mTOR-perturbing molecules to be approved for anticancer use in humans (reviewed in REF. 35). These molecules inhibit mTORC1 through the same mechanism of action as rapamycin, but have different pharmacokinetic and solubility properties that increase their desirability for clinical use. In contrast to mTORC1, FKBP12–rapamycin cannot bind directly to mTORC2 (REFS 29,30), suggesting that the effects of rapamycin on cellular signalling are due to inhibition of mTORC1. A potentially important wrinkle in this seemingly closed story has recently emerged36. It turns out that prolonged treatment with rapamycin — clearly a situation that is relevant to its use in patients — perturbs mTORC2 assembly and, in about 20% of cancer cell lines, the drop in intact mTORC2 levels is sufficient to strongly inhibit Akt signalling (FIG. 2). The binding of FKBP12–rapamycin to mTOR seems to block the subsequent binding of the mTORC2-specific components rictor36 and mSin1 (REF. 37) but it is unknown why in certain cell types rapamycin only partially inhibits mTORC2 assembly. No absolute correlation exists between the tissue of origin of a cell line and the sensitivity of mTORC2 formation to rapamycin, although many cell lines with this property are derived from the haematological system. Recent work provides the first evidence that mTORC2 function can be rapamycin-sensitive in patients. In more than 50% of patients with acute myeloid leukaemia, rapamycin or an analogue inhibited Akt phosphorylation in primary leukaemic cells and the inhibition correlated with the loss of intact mTORC2 (M. Konopleva, personal communication).

So, rapamycin and its analogues are universal inhibitors of mTORC1 and S6K1, and cell-type specific inhibitors of mTORC2 and Akt. As the inhibition of mTORC2 by rapamycin is time and dose dependent38,39, Akt activity in tumours will vary with the length of rapamycin treatment and the dosing regimen (FIG. 2). It is important to keep in mind that, because inhibition of mTORC1 and mTORC2 will not always occur at the same time, markers of mTORC1 inhibition, such as loss of phosphorylated S6, will not necessarily reflect mTORC2 activity. As discussed below, the capacity to sometimes inhibit mTORC2 might help explain why the cellular effects of rapamycin vary among cancer cell lines. Moreover, in a tumour this inhibition might have the beneficial effect of preventing the activation of Akt, through inhibition of S6K1 (FIG. 1), that rapamycin would otherwise cause.

**Anticancer uses for mTOR inhibitors**

Rapamycin and its analogues can inhibit several processes that are relevant to the anti-tumour properties that these molecules exert in pre-clinical cancer models, including cell proliferation, cell survival and angiogenesis (reviewed in REF. 35). Exactly how mTORC1 inhibition mediates all these varied effects is not well worked out and the potential for rapamycin to inhibit mTORC2 and Akt provides additional mechanisms to consider. A case in point is the effects of rapamycin on apoptosis, which vary depending on which cell line is tested. There are many reports of rapamycin promoting pro-apoptotic stimuli36–39 but there are also reports of it promoting cell survival40. As rapamycin universally inhibits the mTORC1 pathway, its effects on apoptosis might correlate with its varying effects on Akt, a well-known regulator of cell survival. In cells in which the drug inhibits mTORC2 and Akt it might promote apoptosis, as has been shown39. On the other hand, when the drug does not inhibit mTORC2, so that mTORC1 inhibition leads to Akt activation, the drug might protect against apoptosis. As induction of apoptosis rather than cytostasis is increasingly considered a prerequisite for an effective anticancer agent, it will be crucial to understand when rapamycin has such effects and where it does not, and to learn how to trigger apoptosis with additional therapies.

Despite the substantial pre-clinical data indicating that rapamycin and its analogues have anti-tumour effects and that mTOR participates in many cancer-related pathways, these molecules have not shown universal anti-tumour activity in early clinical trials. Response rates vary among cancer types from a low of less than 10% in patients with glioblastomas64,65 or advanced renal-cell cancer46 to a high of around 40% in patients with mantle-cell lymphoma (MCL; an aggressive non-Hodgkin lymphoma with a poor prognosis)49. Many in the community have found these results disappointing, but until we understand why rapamycin analogues do have significant anti-tumour effects in certain patients it is too early to draw a conclusion on the utility of inhibiting mTOR in cancer treatment. Clearly, we require more information on which combination of
molecular lesions is likely to make a tumour susceptible to mTOR inhibition. As discussed below, good scientific reasons are emerging as to why rapamycin might benefit particular tumour types, and the hope is that with the proper insights this drug or other mTOR inhibitors might be used for patient benefit. TSC. A strong scientific rationale exists for the use of rapamycin and its analogues in the treatment of TSC. Rapamycin suppresses the molecular consequences of TSC1–TSC2 loss on the mTORC1 pathway and, in cultured cells and model organisms, the drug also reverses the increase in cell size that is a hallmark of the disease (reviewed in Ref. 35). In a recent clinical trial rapamycin reduced the sizes of the astrocytomas that are frequently seen in patients with TSC64, providing the first human data that supports the widely held expectation that rapamycin will be a useful drug for TSC. Because mTORC1 is at least two molecules downstream of the TSC1–TSC2 complex it is unlikely that rapamycin will reverse all TSC-associated phenotypes. It is known that TSC1–TSC2 has targets besides Rheb51,52 and it is likely that Rheb has targets in addition to mTORC1 (REFS 55–57), potentially allowing TSC1–TSC2 loss to cause many mTORC1-independent sequelae. There is already evidence that this is the case as rapamycin cannot reverse the dendritic-spine elongation that is seen in neurons that lack TSC2 (Ref. 58), or the resistance of TSC2-null fibroblasts to hypoxia-induced apoptosis65. The role of mTORC1-independent pathways in disease pathogenesis remains to be determined but will surely be a topic of great interest if not all clinical features of TSC prove sensitive to mTORC1 inhibitors.

Tumours with activated PI3K–Akt signalling. Data from cancer cell lines in vitro and from xenografts indicate that a strong correlation exists between the antiproliferative effects of the rapamycin analogues and the loss of PTEN60,61. Although this correlation is not perfect, work in mouse models bolsters the idea that rapamycin might be particularly effective against tumours with an activated PI3K–Akt pathway. Rapamycin or an analogue blocked both prostate intraepithelial neoplasia62 and the lymphoproliferative disease63 that is caused by expression of an activated allele of Akt. These findings indicate that tumorigenesis that is driven by a hyperactive PI3K–Akt pathway requires the activation of mTORC1 by Akt. Unfortunately, the situation is not as straightforward in patients because rapamycin analogues have not shown good anti-tumour activity against tumours that are known to have high Akt activity, such as glioblastomas46,47 and breast cancers48.

An interesting hypothesis is emerging as to why this might be. As described earlier, by inhibiting mTORC1 rapamycin and its analogues are expected to strongly activate Akt, a prediction that has now been observed in many cancer cell lines in vitro65,66 and in tumours in patients66. Inhibition of PI3K signalling blocks rapamycin-mediated activation of Akt in cancer cells46,47, suggesting a possible strategy for boosting the anti-tumour efficacy of mTORC1 inhibitors45–47. Consistent with this idea, the combination of

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rapamycin and an inhibitor of IGF1R (insulin-like growth factor 1 receptor) prevents Akt activation in various human cancer lines and has a greater antiproliferative effect than rapamycin alone. Similar antiproliferative effects occur in multiple glioma cell lines that have been treated with PI-103 (REF. 67), which is a molecule that inhibits the kinase activity of both mTOR and PI3K p110α (the isoform of the PI3K catalytic subunit that activates Akt in response to insulin). Even though PI-103 inhibits mTORC1, mTORC2 and PI3K p110α, it has anti-tumour activity in mice without overt toxicity.

In cancer cells in which rapamycin inhibits both mTORC1 and mTORC2, the drug inhibits Akt instead of activating it. This phenomenon seems to occur in only a minority of cancer lines and perhaps many of the tumours that do respond to rapamycin monotherapy have drug-sensitive mTORC2 activity and depend on PI3K–Akt signalling. To test this hypothesis it will be necessary to develop biomarkers that predict in which tumours rapamycin will inhibit Akt and to understand the molecular mechanisms that confer this phenotype. Because rapamycin-mediated inhibition of mTORC1 activates the PI3K–Akt pathway, the relative strength of this activation versus the degree of Akt suppression that is caused by inhibition of mTORC2 assembly might set the ultimate levels of Akt activity in a rapamycin-treated cell. Of course, it is probable that the insensitivity of certain tumours to rapamycin does not depend on the inherent sensitivity of mTORC2 assembly to the drug. Rather, as yet unidentified mutations in tumour cells might determine how important mTORC1 signalling is to the proliferation and survival of a particular cancer cell.

**Figure 2** Two models to explain the varying effects of long-term rapamycin treatment on Akt activity. a In this scenario, the assembly of mTORC2 (mammalian target of rapamycin complex 2) is completely sensitive to rapamycin treatment — 24 hours after rapamycin addition no intact mTORC2 remains in the cell. Therefore, Akt (also known as protein kinase B) phosphorylation does not occur and its activity drops. After 1 hour of rapamycin treatment the drug inhibits only mTORC1. This eliminates the inhibitory signal that is normally mediated by S6K1 (ribosomal S6 kinase 1) to IRS1 (insulin receptor substrate 1), which suppresses the activity of the PI3K (phosphatidylinositol 3-kinase)–Akt pathway. Therefore, Akt activity increases with short rapamycin treatment times but is inhibited by prolonged treatment. b In this scenario, Akt activity also increases after 1 hour of treatment but mTORC2 assembly is not completely sensitive to rapamycin, so some mTORC2 remains intact even with prolonged treatment and Akt activity remains at increased (shown) or at baseline (not shown) levels. Only about 20% of cancer cell lines seem to have mTORC2 assembly that is completely sensitive to rapamycin. The size of the icons that represent S6K1 and Akt depicts their activity at different times after rapamycin treatment. FKBP12, intracellular receptor for rapamycin.
lack the tumour suppressor VHL (von Hippel Lindau), which normally inhibits HIF1α, are particularly sensitive to rapamycin in culture and in tumour xenografts7. The expression in the cancer cells of a HIF1α mRNA that is engineered to make its translation resistant to rapamycin can largely eliminate the sensitivity of the cells to the antiproliferative effects of the drug7. In this case, rapamycin is clearly functioning through its action on the cancer rather than endothelial cells.

**Tumours with cyclin D1 overexpression.** Tumours with cyclin D1 overexpression. Tumours with cyclin D1 overexpression.

A hallmark of MCL is the translocation-induced overexpression of cyclin D1 (reviewed in REF. 81). The mTORC1 pathway positively regulates cyclin D1 transcription, translation and stability in many types of cancer cell18,19. Despite the clear rationale for the use of rapamycin in MCL, in tissue culture experiments the drug had the unexpected effect of arresting MCL cells without decreasing their high levels of cyclin D1 (REF. 87). Recent work reveals that hyperactive PI3K–Akt signalling (in some cases caused by PTEN loss) occurs in about 50% of MCLs and it is tempting to speculate that rapamycin might not permit a single molecule to inhibit both mTORC1 and mTORC2. Of course, this will depend on its kinase activity as mTOR might not be the crucial target. To identify compounds that perturb the mTOR activity, many groups are testing because all molecules that inhibit the mTOR kinase domain, the assumption will be molecules that directly inhibit the mTOR complexes through mechanisms other than inhibition of mTOR, it is exciting that this time is finally here.

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