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News and Commentary

Mammalian Target of Rapamycin (mTOR): Pro- and Anti-Apoptotic

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The intracellular receptor of rapamycin, a macrolide antibiotic produced by *Streptomyces hygroscopicus*, is FKBP12 (FK506-binding protein). The rapamycin-FKBP12 complex, in turn, specifically interacts with the mammalian target of rapamycin (mTOR), to potently inhibit mTOR signaling to downstream targets. Cumulative evidence supports the hypothesis that mTOR acts as a master switch of cellular catabolism and anabolism. In addition, mTOR has been recently found to have profound effects on the control of apoptosis.

MTOR is a serine/threonine kinase which signals to downstream effectors, either through direct phosphorylation or via the inhibition of the phosphatase PP2A.[1] Under normal circumstances, in the presence of growth factor and nutrients, mTOR is constitutively activated. This activation is achieved, in part, through the insulin receptor or insulin-like growth factor receptor pathways, via a cascade that involves the activation of phosphatidylinositide-3-kinase (PI3K), then phosphatidyl inositol-3,4,5 phosphate-mediated activation of Akt/PKB-mediated phosphorylation of mTOR.[1] Deacetylated tRNA species accumulating as a result of amino acid shortage may act as negative regulators of mTOR,[2] through a pathway that remains to be elucidated. Moreover, the c-Abl protein tyrosine kinase phosphorylates mTOR and inhibits its action[3] (Figure 1).

Control of translation and autophagy

mTOR phosphorylates and activates the ribosomal S6 kinases (S6K1 and S6K2), which are required for the translation of a group of mRNAs possessing a 5′ terminal oligopyrimidine tract (5′TOP). In addition, mTOR phosphorylates and inactivates the binding protein of eukaryotic translation initiation factor 4E (4E/BP), thereby facilitating 4E-mediated translation of mRNA species possessing a 5′ 'cap'.[1] Moreover, mTOR may indirectly (via S6K which inhibits an inhibitory kinase) induce the dephosphorylation of eukaryotic elongation factor eEF2, thereby facilitating its participation in translation.[4] Altogether, these effects imply that mTOR increases protein synthesis. In addition, mTOR reduces the degradation of proteins. In yeast, TOR inhibits autophagy at two distinct levels. On one hand, mTOR favors the hyperphosphorylation of a factor, Apg13 which, when phosphorylated, cannot activate the autophagy-stimulatory Apg1 kinase. On the other hand, mTOR represses the expression of the AUT7 gene, thereby controlling the size of the autophagosomal compartment.[5] As a net outcome, inhibition of mTOR by carbon or nitrogen starvation or by rapamycin stimulates autophagy. In mammalian cells, autophagy can be inhibited by S6K, and the inhibition of autophagy by amino acids can be prevented by rapamycin treatment.

Of note, it is possible that the mTOR pathway becomes inactivated during apoptosis, at least in some cases. Thus, treatment of 3T3 or Rat-1 cells with four different apoptosis inducers (etoposide, cisplatin, mitomycin C, and staurosporin) leads to dephosphorylation of the two mTOR downstream targets S6K and 4E-BP1.[6,7] These alterations occur in a caspase-independent fashion and may determine the apoptotic arrest of translation.[6,7] Although formal proof in favor of this hypothesis is missing, it is tempting to speculate that the (caspase-independent) cell death-linked increase in autophagy[8] may also be secondary to the inactivation of mTOR and S6K.

mTOR as an apoptosis inhibitor: when rapamycin or its analogs induce apoptosis

Rapamycin alone can induce apoptosis in a cell type-specific fashion. Thus, it kills dendritic cells yet has no cytotoxic effect on monocytes and macrophages.[9] This effect may well contribute to the well established immunosuppressive effect of rapamycin that generally has been linked to its antiproliferative effects on T cells. Rapamycin can sensitize cancer cells to apoptosis induction by cis-platin.[10] Rapamycin has also been reported to selectively kill mouse embryo fibroblasts which are deficient either in p53 or in p21.[11] Recently, it has been found that the oncogenic transformation of human cells induced by either PI3K or Akt is inhibited by rapamycin. Rapamycin fails to reduce transforming activity of 11 other oncoproteins indicating that this effect is specific for the PI3K-Akt pathway.[12] In line with this interpretation, rapamycin antagonizes tumor growth induced by loss of the PI3K antagonist PTEN. $PTEN^{+/-}$ mice spontaneously develop neoplasia, associated with loss of the normal PTEN allele and an increased activation of Akt/PKB and S6K. *In vivo* treatment of such mice with CCI-779, a rapamycin analog, normalizes S6K activity and reduces neoplastic proliferation.[13] Similarly, PTEN-deficient human tumors are more sensitive to CCI-779-mediated growth inhibition than PTEN-expressing cells.[14] This growth inhibition involves both a decrease in proliferation

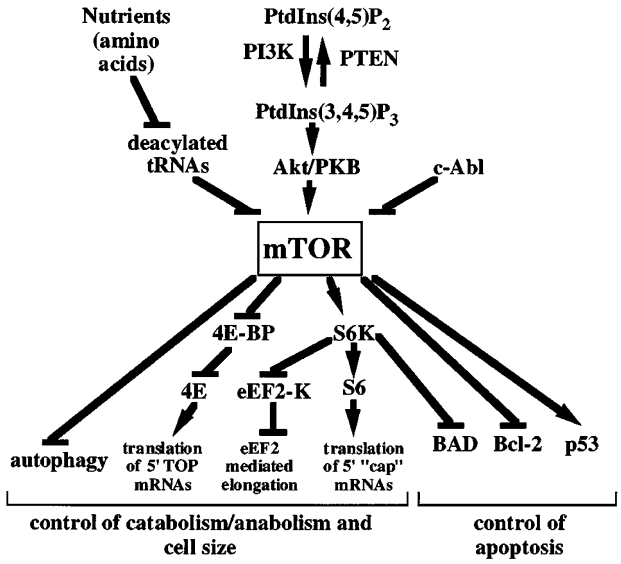

Figure 1 Regulation of mTOR. In the upper half of the figure, some of the mTOR regulatory pathways are shown. The lower part of the figures enumerates some of the downstream targets of mTOR involved in the regulation of protein synthesis and in the control of apoptosis. For details consult main text

and an increase in apoptosis.[14] As a result, rapamycin, as well as its analogs, may become useful for the treatment of some classes of cancer. How does the mTOR inhibit apoptosis? As a possibility, its downstream target S6K, which can bind to mitochondrial membranes, can phosphorylate the pro-apoptotic molecule BAD on serine 136, a reaction which disrupts BAD's binding to the mitochondrial death inhibitors Bcl-XL and Bcl-2 and thus inactivates BAD.[15]

mTOR as an apoptosis inducer: when rapamycin inhibits apoptosis

Rapamycin inhibits the death of syncytia arising from the interaction between cells expressing the HIV-1 envelope (Env) and CD4/CXCR4.[16] In this paradigm, mTOR translocates from the cytoplasm to the nucleus shortly after syncytium formation. Once in the nucleus, it causes phosphorylation of p53 on serine 15, resulting in its transcriptional activation, induction of pro-apoptotic proteins such as Bax, and activation of the mitochondrial cell death pathway.[16–18] Rapamycin inhibits the phosphorylation of p53 and subsequent apoptosis, both in syncytia arising by the co-culture of Env- or CD4/CXCR4-expressing cells and in syncytia arising by the co-culture of Env- or CD4/CXCR4-expressing cells and in syncytia induced by infection with HIV-1.[16] Rapamycin inhibits the phosphorylation and activation of S6K induced by UVB[19] or UVA irradiation.[20] Similarly, UV-elicited p53 phosphorylation has been reported to involve mTOR.[21] Rapamycin also inhibits the taxol-induced apoptosis of human B-cell lines, and taxol can activate mTOR.[22] The opposing effects of rapamycin and taxol may be correlated with an (mTOR-mediated?) phosphorylation/inactivation of Bcl-2.[22] Rapamycin may also inhibit apoptosis induction by TNF in HL-60 cells, although the mechanism of this cytoprotective effect remains elusive.[23] Finally, rapamycin can inhibit hybridoma cell death in bioreactors, thereby increasing the production of monoclonal antibody.[15]

mTOR as a pleiotropic apoptosis regulator – a link between cellular atrophy, apoptosis and autophagic cell death?

The results discussed above suggest that, in addition to its preponderant role in the control of net protein synthesis (and cell size), mTOR may have a pleiotropic function in the regulation of cell death. This function appears to be dictated by the cellular context (cell type and activation state) as well as by multiple downstream targets including well known apoptosis-regulatory proteins such p53, Bad and Bcl-2 (see above, Figure 1). Intriguingly, mTOR can act on a range of additional proteins with potential apoptosis-modulatory functions: protein kinase C (PKC) ε (which is dephosphorylated in a rapamycin-sensitive manner),[24] PKC α and δ (which are phosphorylated and activated),[25] retinoblastoma protein (which is phosphorylated),[26] STAT3 (which is phosphorylated)[27] and c-Myc (which is induced at the transcriptional level),[25] thereby adding further elements to the puzzle. Future investigations will unravel which among these targets determine the pro- or anti-apoptotic *modus operandi* of mTOR. Likewise, it will be passionate to learn to which extent mTOR acts as a molecular link between atrophy, apoptosis, and autophagic cell death.

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