# Mechanistic / mammalian target protein of rapamycin signaling in hematopoietic stem cells and leukemia



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# Mechanistic/mammalian target protein of rapamycin signaling in hematopoietic stem cells and leukemia

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**Mechanistic/mammalian target protein of rapamycin (mTOR) is an evolutionarily conserved kinase that plays a critical role in sensing and responding to environmental determinants such as nutrient availability, energy sufficiency, stress, and growth factor concentration. mTOR participates in two complexes, designated mTOR complex 1 (mTORC1) and 2 (mTORC2), both of which phosphorylate multiple substrates. Recent studies have revealed that the fine-tuning activity of mTOR complexes contributes to both maintenance of hematopoietic stem cells (HSCs) and suppression of leukemogenesis. Dysregulation of mTORC1 activity results in impaired HSC homeostasis. Abnormalities of mTOR signaling are observed in many patients with leukemia and genetic studies clearly show that the leukemogenesis associated with *Pten* deficiency involves both mTORC1 and mTORC2. Although the several mTOR inhibitors have been developed for cancer therapy, effectiveness of the inhibitors for eradication of leukemia stem cells (LSCs) is unknown. Advances in understanding of how mTOR signaling is involved in mechanisms of normal HSC and LSC homeostasis may lead to novel therapeutic approaches that can successfully eradicate leukemia. (*Cancer Sci* 2013; 104: 977–982)**

Improved cell purification and transplantation techniques have contributed to the identification of cell populations known as leukemia-initiating cells, leading to the idea that leukemias are organized as hierarchies of leukemia cells that are sustained by rare leukemia stem cells (LSCs).[1] Previous studies have suggested that shared mechanisms regulate stem cell properties (stemness) in both hematopoietic stem cells (HSCs) and LSCs,[2] leading to the hypothesis that LSCs may originate from HSCs. Several lines of evidence indicate that chronic myelogenous leukemia (CML) originates from HSCs that have sustained the chromosomal translocation.[3] On the other hand, recent findings have suggested that acute myeloid leukemia (AML) can originate from myeloid progenitors and acquire stemness properties during leukemogenesis.[4–7] Despite the differing origins of LSCs among different leukemias, there appears to be a common regulatory mechanism governing "stemness" and thus the behavior of HSCs and LSCs.

A current topic in the stem cell research field is the regulation of HSC homeostasis by the fine-tuning of mechanistic/mammalian target protein of rapamycin (mTOR) signaling. mTOR is an evolutionarily conserved kinase that plays a critical role in sensing and responding to environmental determinants such as nutrient availability, energy sufficiency, stress, and growth factor concentration.[8,9] Because HSCs reside in a hypoxic bone marrow environment and use glycolysis, rather than mitochondrial oxidative phosphorylation, to meet their energy demands,[10] molecules used in regulation of cellular metabolism such as mTOR are vital for HSC homeostasis. In addition, recent studies have demonstrated that the activation of mTOR complexes also regulates the leukemogenesis. Understanding the roles of mTOR signaling in stemness of leukemias is important because mTOR inhibitors have been developed for cancer therapy. In this article, we focus on the molecular aspects of how mTOR signaling may be involved in the homeostasis of normal HSCs and LSCs.

## mTOR Signaling Pathways

**Regulation of mTOR complex 1 activity.** mTOR is a serine/threonine protein kinase that belongs to the phosphoinositide-3-kinase (PI3K)-related kinase family. mTOR participates in two complexes, designated mTOR complex 1 (mTORC1) and 2 (mTORC2),[8,9] both of which phosphorylate multiple substrates. mTORC1 consists of mTOR, regulatory-associated protein of mTOR (Raptor), mammalian lethal withsec-13 protein 8 (mLST8/GβL), DEP domain-containing mTOR-interacting protein (DEPTOR), 40 kDa Pro-rich AKT substrate (PRAS40; also known as AKT1S1), and the Tel2-interacting protein (Tti1)/Tel2 complex (Fig. 1).

mTORC1 activation is induced by growth factors, cytokines or insulin. Activation of PI3K via receptor tyrosine kinases (RTKs) in response to these stimuli leads to phosphorylation of phosphatidylinositol-4,5-bisphosphate (PIP2) to form PIP3. This increase in PIP3 recruits AKT (also known as protein kinase B [PKB]) to the membrane, where it is phosphorylated by phosphoinositide-dependent kinase 1 (PDK1). One of the substrates of AKT is tuberous sclerosis 2 (TSC2; also known as tuberin). A heterodimer of TSC2 and TSC1 (also known as hamartin) exhibits GTPase-activating protein (GAP) activity towards Ras homolog enriched in brain (Rheb), converting it to the inactive GDP bound form. Because GTP-loaded Rheb is vital for the activation of mTOR kinase activity,[11,12] and AKT-mediated phosphorylation of TSC2 blocks TSC's inhibition of Rheb activity, AKT effectively activates mTORC1 through the TSC complex. AKT also activates mTORC1 in a manner independent of the TSC1/TSC2-Rheb pathway, because AKT-induced phosphorylation of PRAS40 results in its dissociation from mTORC1.[13–16] In addition to the PI3K-AKT pathway, mTORC1 can be activated by extracellular-signal-regulated kinase 1/2 (ERK1/2)-ribosomal S6 kinase (RSK1) pathway, mediated with TSC1/TSC2 complex.[17] WNT can also stimulate mTORC1 because WNT inactivates glycogen synthase kinase 3β (GSK3β), which phosphorylates TSC2.[18]

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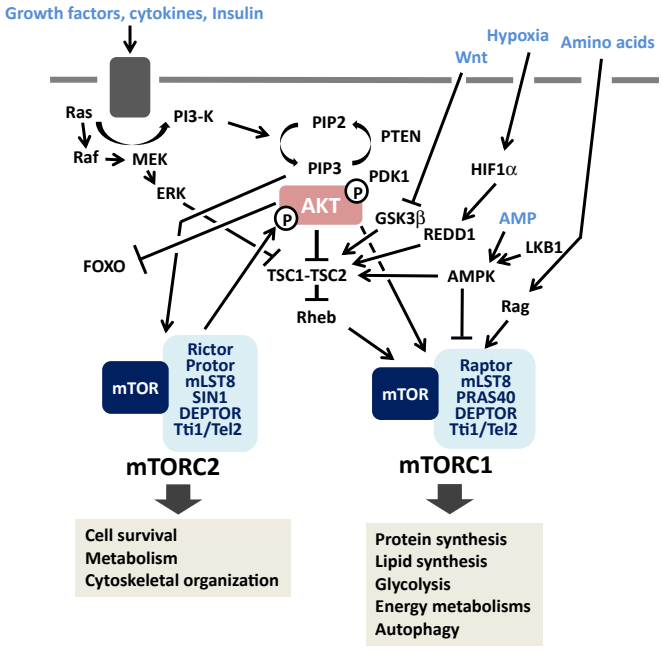


**Fig. 1.** mTOR signaling pathways. mTOR associates with the indicated molecules to form the mTORC1 and mTORC2 complexes. mTORC1 activity is regulated by a variety of determinants, including growth factors, cytokines, amino acids, hypoxia and energy sources. Numerous signaling molecules, including PI3K, AKT, RAS, TSC1/2, Rheb, AMPK, HIF1α, GSK3β, and Rag, are involved in the regulation of mTORC1 activity, which controls protein synthesis, lipid synthesis, glycolysis, energy metabolism and autophagy. Although much less is known about mTORC2, PI3K signaling reportedly stimulates the activity of this complex, which influences cell survival, metabolism, and cytoskeletal organization.

As well as by the above growth factor signaling pathways, mTORC1 activity is influenced by changes in intracellular energy and oxygen conditions. For example, the essential energy sensor AMP-activated protein kinase (AMPK) can regulate mTORC1 activity. Under conditions of glucose starvation, AMP levels rise and AMPK activity increases in a manner dependent on the tumor suppressor liver kinase B1, resulting in AMPK-mediated phosphorylation of TSC2 and mTORC1 inactivation.[19] AMPK can also directly phosphorylate Raptor, again inhibiting mTORC1.[20] Under conditions of hypoxia, hypoxia-inducible factor 1α (HIF1α) is upregulated and induces DNA damage response 1 (REDD1), which enhances the activity of the TSC complex and inhibits mTORC1.[21,22]

Amino acids, particularly leucine and glutamine, are important regulators of mTORC1 activation. A member of the mammalian Rag small GTPase family, such as RagA or RagB, interacts with RagC or RagD to form a heterodimeric complex that is constitutively anchored on the surface of the lysosome.[23,24] Upon amino acid deprivation, RagA or RagB becomes loaded with GDP and unable to recruit mTORC1 to the lysosome surface which is a location for mTORC1 activation.[25]

**Targets of mTORC1.** mTORC1 controls multiple cellular processes, including protein synthesis, glucose metabolism, fatty acid and sterol synthesis, mitochondrial biogenesis, and autophagy.

*Protein synthesis.* The assembly of the eukaryotic translation initiation factor 4F (eIF4F) complex on the 5′ cap structure of mRNA is required for initiation of translation.[8] This interaction is controlled by 4EBP1 (inhibitory 4E-binding protein 1; also known as eIF4EBP1). When hypophosphorylated, 4EBP1 binds tightly to eIF4E and prevents it from interacting with eIF4G. Phosphorylation of 4EBP1 by mTORC1 leads to its dissociation from eIF4E, allowing the recruitment of eIF4G to the 5′ cap and translation initiation. Another set of direct targets of mTORC1 in this context are the p70 ribosomal protein S6 kinases (p70S6Ks). S6Ks phosphorylate many proteins, including ribosomal protein S6 (RPS6), eukaryotic translation elongation factor 2 kinase (eEF2K), cap-binding protein 80 (CBP80), S6K1 Aly/REF-like substrate (SKAR), eukaryotic translation initiation factor 4B (eIF4B), and programmed cell death 4 (PDCD4). mTORC1-mediated phosphorylation of these molecules leads to stimulation of mRNA biogenesis as well as translation initiation and polypeptide elongation. mTORC1 controls the translation of a set of genes that possess a 5′ terminal oligopyrimidine tract (5′ TOP). Many of these genes encode ribosomal proteins or translation elongation factors.

*Lipogenesis.* mTORC1 is required for AKT-dependent lipogenesis, and a key set of targets involved in this process are sterol regulatory element-binding proteins (SREBPs).[26] As master transcriptional regulators of lipid metabolism, SREBPs control the expression of genes encoding enzymes, including acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), and stearoyl-CoA desaturase 1 (SCD-1), required for fatty acid and cholesterol biosynthesis.[27] mTORC1 regulates SREBP by controlling the nuclear entry of lipin 1, a phosphatidic acid phosphatase.[28] Dephosphorylated, nuclear, catalytically active lipin1 promotes nuclear remodeling and mediates the effects of mTORC1 on SREBP target genes. In addition, mTORC1 induces the expression of peroxisome proliferator-activated receptor-γ (PPAR-γ), which is mediated by CCAAT/enhancer binding protein-β (C/EBP-β), C/EBP-δ and C/EBP-α. Induction of PPAR-γ results in the upregulation of the uptake, synthesis and esterification of fatty acids and their storage in adipose cells.

*Energy metabolism.* mTORC1 is crucial for several pathways of intracellular energy metabolism. mTORC1 activates the glycolytic flow by HIF1α activation, as well as the mitochondrial biogenesis mediated by PPAR-γ coactivator 1α (PGC1α) and the transcription factor Ying-Yang 1 (YY1).[29,30] Activation of both pathways contributes to elevation of ATP levels. In contrast, inhibition of mTORC1 activates autophagy, a system that degrades proteins to generate energy for survival under conditions of stress or starvation.[31] An interaction between Raptor and the autophagy-related gene 1(Atg1)/UNC-51-like kinase 1 (ULK1) kinase mediates the incorporation of mTORC1 into the ULK1-Atg13-FAK family-interacting protein of 200 kDa (FIP200) complex, which is required for autophagy. mTOR can phosphorylate Atg1/ULK1 and Atg13, suggesting that autophagy is directly regulated by mTORC1.[32]

**Feedback loop by mTORC1 inhibition.** To identify new proteins whose phosphorylation is regulated by mTOR, large-scaled phosphoproteomics analyses have been performed.[33,34] Such studies have provided valuable information about the existing and novel mTOR target molecules, including those involved in negative feedback loops. mTORC1 activation suppresses the PI3K-AKT pathway in a negative feedback loop. A known mechanism underlying this loop is the destabilization of insulin receptor substrate-1 (IRS-1) by phosphorylated S6K1. However, growth factor receptor-bound protein 10 (Grb10) was identified as a new target of mTORC1 in this context through these studies.[33,34] It had been previously reported that Grb10 negatively regulates growth factor signaling by binding to the insulin and insulin-like growth factor 1 (IGF-1) receptors. The phosphoproteomics studies revealed that mTORC1-mediated phosphorylation stabilizes Grb10, leading to feedback inhibition of the PI3K. Grb10 also contributes to the suppression of tyrosine phosphorylation of IRS1 and IRS2, and to p85 PI3K recruitment by IRSs. In addition, since Grb10 is frequently downregulated in a range of cancers,


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Grb10 may be a tumor suppressor that is regulated by mTORC1. Thus, identification of feedback signals can open up new areas of investigation in mTOR biology.

**mTOR complex 2.** The mTORC2 consists of mTOR, Rapamycin-insensitive companion of mTOR (Rictor), mLST8, SAPK-interacting protein (SIN1), DEPTOR, Tti1/Tel2 and protein observed with Rictor (Protor) (Fig. 1). mTORC2 has not been as well studied as mTORC1, and although insulin signaling is known to stimulate mTORC2, the mechanism is undefined. It was recently demonstrated that insulin-induced PI3K signaling stimulates an interaction between mTORC2 and ribosomes that promotes mTORC2 activation.[35] Activated mTORC2 phosphorylates the AGC kinases, which are named for protein kinases A, G and C. Known substrates of mTORC2 are AKT (S473, T450), serum/glucocorticoide-regulated kinase 1 (SGK1), and PKCα. At the cellular level, mTORC2 regulates cell shape by phosphorylating and activating PKC-α, which influences the actin cytoskeleton.[36,37] It has been reported that mTORC2 inactivation leads to a failure in the phosphorylation of AKT(S473), which in turn leads to the activation of the Forkhead box class O transcription factor (FoxO) 1/3a transcription factors, while other AKT targets, TSC2 and GSK3, and the TORC1 effectors, S6K and 4E-BP1, are not affected.[38,39] Further studies regarding upstream and downstream signals are needed to understand physiological roles of mTORC2.

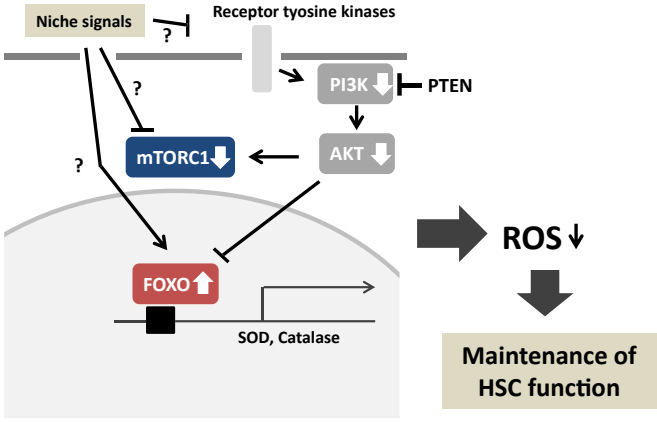


**Fig. 2.** mTOR signaling in the maintenance of HSC functions. In quiescent HSCs within the BM niche, PI3K-AKT signaling is suppressed. mTORC1 is inactivated and FoxO transcription factors are activated. The transcription of antioxidant genes such as superoxide dismutase (SOD) and catalase can proceed. Failure of mTORC1 suppression or FoxO inactivation results in an increase in ROS levels, leading to HSC dysfunction. It remains unclear how mTORC1 and FoxOs are regulated by signals emanating from the BM niche *in vivo*.

## mTOR Signaling in HSCs

**Downregulation of mTORC1 is essential for HSC homeostasis.** Within their bone marrow (BM) niche, HSCs adopt a quiescent state and remain in the non-dividing G0 phase of the cell cycle until the generation of additional HSCs/progenitors is required. This quiescence of HSCs is also critical for the maintenance of their self-renewal capacity, which is essential for the maintenance of appropriate HSC numbers.[40] AKT is highly phosphorylated and activated in most hematopoietic progenitor cells but suppressed in freshly isolated HSCs, indicating that the PI3K-AKT pathway is downregulated in the BM niche[41] (Fig. 2). In the absence of growth factor stimulation, FoxO proteins are retained in an active state in the nucleus and induce their transcriptional targets. We and others have shown that FoxOs are essential for the maintenance of quiescence and self-renewal capacity in HSCs *in vivo*.[42–44] Consistent with the suppression of PI3K-AKT signaling observed in HSCs, mTORC1 is downregulated in these cells. Indeed, studies of *Tsc1*-deficient mice have demonstrated that mTORC1 suppression is essential for HSC quiescence.[45,46] In the absence of Tsc1, phospho-S6 accumulates in HSCs, inducing their entrance into the cell cycle. *Tsc1*-deficient HSCs exhibit increased mitochondrial mass and mitochondrial DNA per HSC. The transcription of many mitochondrial genes is elevated, indicating that mTORC1 activation triggers mitochondrial biogenesis. In addition, *Tsc1*-deficient HSCs show increased concentrations of reactive oxygen species (ROS). Interestingly, although *Tsc1* deletion induces a transient increase in HSC numbers in these mutant mice, these cells eventually become exhausted. Suppression of ROS level by treatment with the antioxidant molecule N-acetylcysteine (NAC) can prevent the phenotypic defects of *Tsc1*-deficient HSCs. Thus, suppression of mTORC1 contributes to HSC maintenance by repressing mitochondrial biogenesis.

HSC functions decline with age in mammals in a manner reportedly due to increased mTORC1 activity.[47] In HSCs from aged mice, the phosphorylated forms of S6K, S6 and AKT are significantly elevated. Interestingly, aged mice treated with the mTORC1 inhibitor rapamycin (also known as sirolimus) have an extended lifespan and show restored HSC self-renewal and normal hematopoiesis. A recent study has revealed that rapamycin treatment combined with activation of canonical Wnt–β-catenin signaling maintains murine and human HSCs *ex vivo* and increases the number of long-term HSCs *in vivo*.[48] Thus, a microenvironment in which mTORC1 is suppressed is critical for the maintenance of HSC homeostasis.

**The Abnormal Activation of mTORC1 or mTORC2 Associated with Pten Deficiency Impairs HSC Functions.** Phosphatase and Tensin Homolog (PTEN) is a negative regulator of PI3K-AKT signaling. *Pten* deficiency in mice disrupts HSC quiescence, leading to HSC depletion.[49,50] *Pten*-deficient HSCs show increased phospho-AKT and elevated phospho-S6.[51] Because rapamycin can reverse the defective phenotypes of *Pten*-deficient HSCs,[49] mTORC1 activation is thought to be responsible for HSC depletion. The mechanism of this depletion appears to involve the upregulation of the tumor suppressors p16 and p53 but is not mediated by ROS because NAC treatment did not rescue the defective HSC phenotypes.[51]

mTORC2 is also responsible for the disrupted functions of *Pten*-deficient HSCs.[52] The phosphorylation of AKT(S473), which depends on mTORC2 activity, is increased in HSCs upon *Pten* deletion. Furthermore, deficiency of *Rictor*, an essential component of mTORC2, reduces AKT phosphorylation and restores the abilities of *Pten*-deficient HSCs to undergo accelerated cell cycling and reconstitute the BM.[52]

## Essential Role of mTOR Activation in Leukemogenesis

Abnormalities of mTOR signaling are observed in many patients with leukemia, including those suffering from AML, T cell acute lymphoblastic leukemia (T-ALL), Philadelphia chromosome positive B precursor acute lymphoblastic leukemia ($Ph^+$B-ALL), B cell chronic lymphocytic leukemia (B-CLL), CML, and high-risk myelodysplastic syndromes (MDS).[53,54] Alterations in PTEN, PI3K or AKT are particularly frequent in T-ALL patients.[55] In addition, although genetic aberrations in the PI3K-AKT-mTOR pathway are not found in many AML patients, mTORC1 is often activated. Therefore, it has been suggested that mTORC1 may be activated by a pathway independent of PI3K-AKT, such as the MEK/ERK pathway.

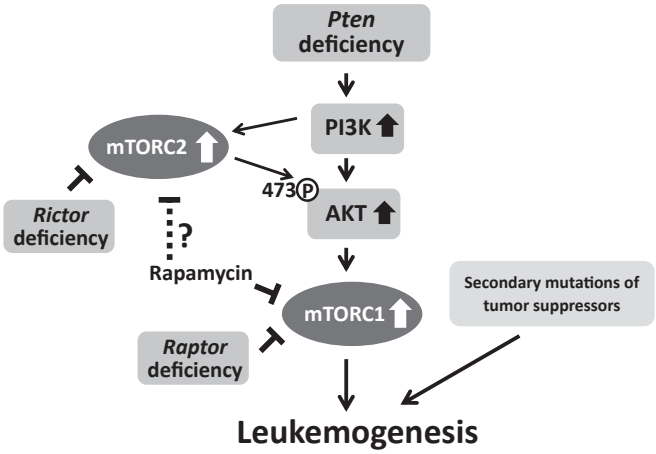


**Fig. 3.** Role of mTOR signaling in leukemogenesis induced by *Pten* deletion. *Pten* deficiency in murine hematopoietic cells promotes myeloproliferative disease that is followed by leukemia development. Thus, leukemogenesis is associated with PI3K-AKT activation in which PI3K activates both mTORC1 and mTORC2. Deletion of either *Raptor* (mTORC1 inactivation) or *Rictor* (mTORC2 inactivation) inhibits leukemia development. Rapamycin, an allosteric mTORC1 inhibitor, suppresses the leukemogenesis, although it might also affect mTORC2. Secondary mutations of tumor suppressors, including p53, may be required for leukemogenesis.

Several groups have used genetically modified mouse models to dissect the role of mTOR in leukemogenesis (Fig. 3). *Pten* deficiency in murine hematopoietic cells promotes myeloproliferative disease followed by leukemia development.[49–51] As mentioned above, since p53 is upregulated in HSCs by *Pten* deficiency and loss of heterozygosity of p53 allele is found in *Pten* deficiency-evoked leukemia,[51] secondary mutations of tumor suppressors may be required for the leukemogenesis. Because these phenotypes are inhibited by rapamycin, mTORC1 activation has been thought to induce leukemogenesis.[49] Alternatively, since prolonged treatment of rapamycin reportedly inhibits not only mTORC1, but also mTORC2, in some cell lines,[56] rapamycin might suppress leukemogenesis via mTORC2 inhibition. Consistent with this hypothesis, *Raptor* deletion significantly prolongs the survival of *Pten*-deficient mice and can ameliorate some of their neoplasia-associated phenotypes.[57] *Rictor* deficiency can also reduce the severity of myeloproliferative disease and leukemia incidence.[52] Since AKT suppression mediated by mTORC2 inactivation may result in suppression of mTORC1 inactivation, it has been suggested that mTORC2 plays a role in leukemogenesis evoked by *Pten* deficiency partly through its effects on mTORC1.[52] Thus, both mTORC1 and mTORC2 play critical roles in leukemogenesis.

## Targeting mTOR Signaling for Leukemia Treatment

**mTOR inhibitors.** Rapamycin (Silolimus) and its derivative "rapalogs", including temsirolimus, everolimus and ridaforolimus, are all allosteric inhibitors of mTORC1. Rapamycin binds to FK506 binding protein 12 (FKBP12) to form a complex that inactivates mTORC1.[8,9] Mice subjected to long-term treatment with rapamycin also show mTORC2 inhibition, although the mechanisms are unclear.[56,58] These effects on mTORC1 have led to the investigation of rapalogs as therapies for tumors, and everolimus and temsirolimus have been approved for the treatment of renal cell carcinomas. With respect to leukemias, several reports have indicated that rapalogs show only a mild effect on AML cells. In a phase I/II clinical trial of everolimus, a possible benefit was observed in only one of five MDS patients and in none of nine AML patients.[59] Similarly, a phase I dose escalation study of rapamycin combined with the MEC (mitoxantrone, etoposide, cytarabine) chemotherapy regimen in patients with relapsed, refractory, or untreated secondary AML showed no evidence of synergy between MEC and rapamycin.[60] A phase II clinical trial of everolimus in patients with previously treated indolent hematologic malignancies resulted in a partial response in four of 22 heavily pretreated CLL patients.[61] A phase II study of ridaforolimus as treatment for 52 patients with hematological malignancies showed that partial responses were achieved in 10% of patients, while hematologic improvement /stable disease was observed in 40% of patients.[62]

The limited ability of rapalogs to exert tumor cell cytotoxicity may be due to the presence of rapamycin-insensitive mTOR substrates such as 4EBP1.[63,64] Adenosine triphosphate (ATP)-competitive mTOR inhibitors are much better inactivators of mTOR target gene phosphorylation. Indeed, the phosphoproteomic analyses cited above have revealed that there are critical differences in the spectrum of targets affected by rapamycin versus ATP-competitive mTOR inhibitors.[33,34] In addition, such

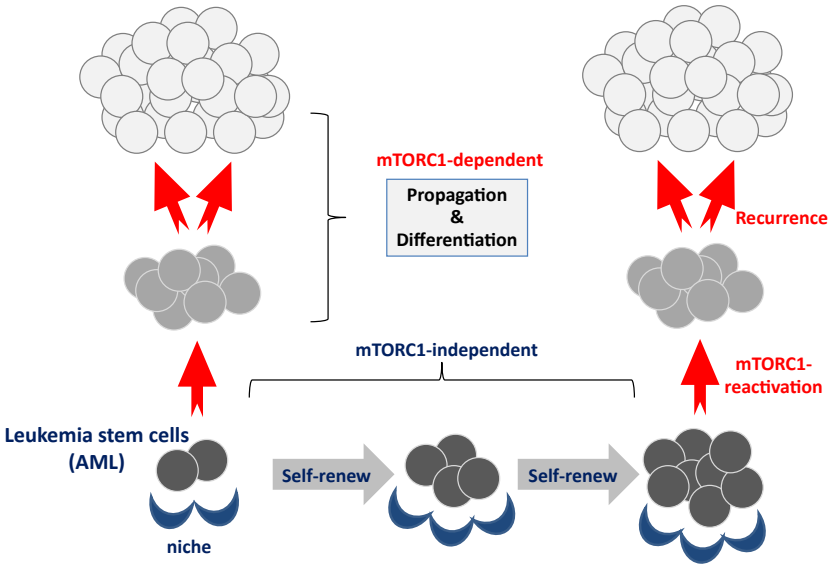


**Fig. 4.** Distinct roles of mTORC1 in differentiated versus undifferentiated leukemia cells in an AML model *in vivo*. Murine AML stem cells in a BM niche can self-renew via a pathway independent of mTORC1, and so can survive when a treatment that blocks mTORC1 is applied. These stem cells cannot differentiate and propagate unless mTORC1. When mTORC1 is reactivated in AML stem cells, the cells can once again differentiate and propagate, causing a recurrence of leukemia. Understanding of the mechanisms by which AML stem cells self-renew should suggest novel therapeutic approaches that will successfully eradicate leukemia in a combination with mTORC1 inhibition.


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agents affect mTORC2 as well as mTORC1. Consistent with these observations, the ATP-competitive mTOR inhibitors PP242[65] and OSI-027[66] have potent anti-leukemic effects. Although a potential drawback to these agents is their inhibition of multiple targets, which could lead to unwanted side-effects or serious damage to normal tissues, these inhibitors are expected to have powerful anti-leukemic effects.

**mTORC1 inhibition by genetic modification in AML.** Although mTORC1 activation can clearly trigger leukemogenesis (as described above), until recently it was not known if targeting mTORC1 could affect the behavior of an established leukemia *in vivo*. To address this question, we generated an MLL-AF9–driven mouse model of AML and induced rapid deletion of the *Raptor* gene once leukemia was established.[67] In AML-bearing mice, *Raptor* deficiency significantly suppressed leukemia progression by inducing the apoptosis of differentiated, but not undifferentiated, leukemia cells. However, a subset of AML cells that had an undifferentiated phenotype and was highly enriched for leukemia-initiating cells was able to survive long-term *in vivo* without mTORC1 activity (Fig. 4). The re-introduction of the *Raptor* gene into these Raptor-deficient AML cells induced them to propagate and differentiate, indicating that although the tumor-propagating properties of AML stem cells are dependent on mTORC1, their self-renewal capacity is not.

The observation that the survival and cell cycling of AML stem cells *in vivo* were not altered by mTORC1 inactivation was puzzling because phosphorylation of mTORC1 targets, including a rapamycin insensitive substrate, 4EBP1, was dramatically suppressed in these cells. Although 4EBP1 phosphorylation reportedly induces cap-dependent protein translation, which controls the growth of individual cells, deletion of *Raptor* had no effect on the cell size of AML stem cells. Since AKT phosphorylation was attenuated in *Raptor* deficient AML cells, it is not the case that AKT activation by a feedback loop of mTORC1 inhibition supports survival of AML stem cells. Microenvironmental factors possibly support mTORC1-independent survival of AML stem cells. In this case, combined therapies with inhibition of both such microenvironmental factors and mTORC1 activity may lead to novel therapeutic approaches that will successfully eradicate AML stem cells. Further dissection of the mechanisms and regulation underlying the mTORC1-independent survival of AML cells in the BM niche is needed.

## Perspectives

In this review, we have focused on recent advances in understanding the roles of mTOR signaling in HSC and LSC biology. The findings we have presented collectively suggest that the mechanisms controlling mTOR activity are critical for the physiological and pathological regulation of both normal and malignant hematopoiesis. Although the roles of mTOR signaling in LSCs are very complicated, LSC behavior may be controlled by similar machinery governing the behavior of normal HSCs and/or hematopoietic progenitors. Therefore, a detailed comparison of mTOR signaling pathways and targets in HSCs and LSCs cells may provide valuable insights useful for overcoming the current limitations of leukemia therapy. Increased understanding of the mechanisms by which LSCs self-renew may point the way to novel therapeutic approaches that can successfully eradicate leukemia.

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## Disclosure Statement

The authors have no conflict of interest.

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