

Akt

A double-edged sword for hematopoietic stem cells

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Abbreviations: PI3K, phosphatidylinositol 3-kinase; AML, acute myeloid leukemia; FLT3-ITD, fms-like tyrosine kinase 3-internal tandem duplication; HSCs, hematopoietic stem cells; *Pten*, phosphatase and tensin homolog; T-ALL, T-cell lymphoblastic leukemia; *FOXOs*, forkhead box, subgroup O transcription factors; *TSC1*, tuberous sclerosis complex 1; mTOR, mammalian target of rapamycin; GFP, green fluorescent protein; LSK, Lin-Sca1⁺c-kit⁺

The phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway is dysregulated in a wide range of malignancies, including leukemia, and several members of this pathway are attractive therapeutic targets in oncology.¹ Although Akt is constitutively phosphorylated in up to 90% of cases of acute myeloid leukemia (AML), its role in AML has been unclear.² In some cases, Akt activation is attributed to activation of tyrosine kinases, such as BCR-ABL or FLT3-ITD, but in many cases the mechanism of Akt phosphorylation and its significance is unknown. Is AKT activation simply a marker of leukemic transformation, or is it a driver of leukemogenesis?

The hematopoietic system has been the primary system for interrogating the cancer stem cell hypothesis, and pioneering work from Bonnet, Dick and others has demonstrated that leukemic stem cells can arise from the transformation of hematopoietic stem cells (HSCs) or myeloid progenitors.³⁻⁵ So what effect does Akt activation have on these primitive cell populations? There are some insights into the roles of PI3K/Akt signaling in HSCs from mouse models in which members of this pathway are knocked out. Conditional deletion in HSCs of *Pten*, a phosphatase which antagonizes PI3K signaling, or the Akt targets FOXO transcription factors and *TSC1*, all result in a myeloproliferative phenotype with depletion of the stem cell pool through accelerated cycling of HSCs⁶⁻⁹ (Fig. 1A). However, only mice

with homozygous *Pten* deletion progress to AML or T-cell lymphoblastic leukemia (T-ALL) (Fig. 1B). Since Akt is the most commonly dysregulated member of the PI3K pathway in human AML, it is important to directly examine the role of Akt in HSCs and in leukemogenesis.

Given that hematopoietic growth factors such as erythropoietin, thrombopoietin, c-kit and Flt3 all activate the PI3K/Akt pathway, it seems likely that Akt activation would have important effects on hematopoiesis. It has been reported that high Akt activity promotes neutrophil and monocyte development, and augments erythropoietin-induced erythrocyte development.¹⁰ However, the role of Akt activation in HSCs has been poorly defined. Using a bone marrow transplant (BMT) system in which wild-type bone marrow cells were transduced with a retrovirus expressing myristoylated Akt1 fused to GFP (myr-Akt-GFP) and transplanted into lethally irradiated recipients, we found that Akt activation in hematopoietic cells can induce myeloproliferative disease and T-ALL, as well as AML at a lower frequency.¹¹ Mice with *Pten* deletion in HSCs develop AML at a much higher rate, highlighting the likely importance of additional downstream targets of Pten loss in addition to Akt. Interestingly, there were notable differences in downstream target activation between different cell types in myr-Akt mice. For example, while increased FOXO1 phosphorylation

was observed in both thymocytes and splenocytes of diseased myr-Akt mice, there were much greater levels of phosphorylated ribosomal protein S6, an mTOR target, in thymocytes than in splenocytes. Interestingly, this correlates with the response to the mTOR inhibitor rapamycin—there was a dramatic decrease in the incidence of T cell lymphoma in myr-Akt transplant recipients leading to increased overall survival, but no change in the incidence of MPD or AML. These results underscore the importance of dissecting specific targets of signaling pathways in each disease cell population. An inhibitor that works on one cell type may not be as effective in a different disease cell type, even if those cells harbor the same upstream molecular lesion.

The myr-Akt BMT system has also revealed the effects of Akt activation on HSCs and myeloid progenitors. Akt decreased the proportion of GFP-positive Lin-Sca1⁺c-kit⁺ (LSK) cells, which include the HSCs, and progenitors in myr-Akt mice. LSK cells were driven into the S/G₂/M stages of the cell cycle, initially causing an expansion of the LSK compartment, then leading to an increase in apoptosis, and finally to depletion of the LSK and progenitor populations. Akt activation was associated with impaired engraftment in vivo and cobblestone formation in vitro. Rapamycin could partially rescue cobblestone formation, suggesting that the mTOR axis is particularly important

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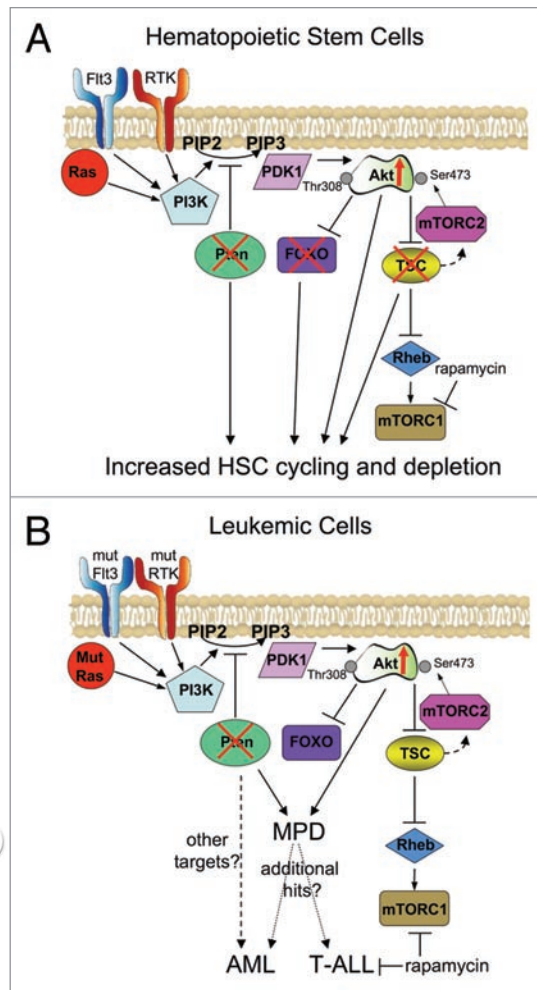


Figure 1. Schematic of PI3K signaling in hematopoietic stem cells (HSCs) (A) and in leukemic cells (B). (A) Activation of Akt or inactivation of Pten, FOXO or TSC1 in HSCs all lead to increased HSC cycling and depletion. (B) Activation of Akt or inactivation of Pten in hematopoietic cells leads to myeloproliferative disease (MPD), AML and T-ALL, likely through cooperation with additional hits. Loss of Pten may also activate additional targets, leading to AML. mut, mutated; RTK, receptor tyrosine kinase.

in HSCs. A similar dependence of HSC homeostasis on mTOR was also observed in mice with *Pten* deletion and *TSC1* deletion.^{6,8} Given what we know about leukemic stem cells, it is surprising that the putative target cell populations for leukemic transformation are the very populations that are depleted by Akt activation. This is difficult to reconcile, but it is tempting to speculate that additional mutations may be required in the stem and progenitor populations in order to overcome the deleterious effects of Akt on these cells and to allow for transformation to occur

(Fig. 1B). This could potentially explain the low frequency of AML (10%) that was observed in the myr-Akt model. One possibility is that a second hit may select for the ability to tolerate higher levels of Akt activation, allowing these cells to survive long enough to become transformed. It remains to be seen what additional perturbations can play this role in HSCs and progenitors. Whether Akt is acting by itself or in concert with other pathways, it clearly appears to be a key perpetrator in the pathogenesis of AML, and not just an innocent bystander.

References

1. Martelli AM, et al. *Expert Opin Investig Drugs* 2009; 18:1333-49.
2. Martelli AM, et al. *Leukemia* 2006; 20:911-28.
3. Cozzio A, et al. *Genes Dev* 2003; 17:3029-35.
4. Huntly BJ, et al. *Cancer Cell* 2004; 6:587-96.
5. Bonner D, et al. *Nat Med* 1997; 3:730-7.
6. Chen C, et al. *J Exp Med* 2008; 205:2397-408.
7. Zhang J, et al. *Nature* 2006; 441:518-22.
8. Yilmaz OH, et al. *Nature* 2006; 441:475-82.
9. Tothova Z, et al. *Cell* 2007; 128:325-39.
10. Buitenhuis M, et al. *Cell Cycle* 2009; 8:560-6.
11. Kharas MG, et al. *Blood* 2009; 115:1406-1415.