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Treating leukemia at the risk of inducing severe anemia

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Abstract

Anemia is a frequently observed adverse effect in cancer patients who receive chemotherapy or drugs designed to block specific oncogenic signaling pathways, although the underlying mechanisms are poorly understood. An article first published online (Zhu HH, Luo X, Zhang K, et al. Proc Natl Acad Sci USA 2015;112:13342–13347) presented data indicating that cell type-specific pathway cross-talk is likely an important mechanism to consider. Shp2 and Pten, two master regulators of central cytoplasmic signaling pathways, oppose each other in myeloproliferation and leukemogenesis, but cooperate in promoting erythropoiesis. Thus, genetic ablation or pharmacologic inhibition of Shp2 suppresses the leukemogenic effect of Pten loss, yet simultaneously induces severe anemia in mice with Pten deficiency in blood cells.

Many current cancer therapeutic efforts focus on targeting specific intracellular signaling pathways that are believed to drive the pathogenesis of malignancies. Development of targeted therapy has benefited largely from the discovery and exploration of specific signaling components, especially enzymes or pathways driving the disease processes. However, the lack of understanding of signal cross-talk hinders therapeutic approaches. This is a major reason why so many drugs, although designed to target elucidated disease mechanisms, fail in clinic trials because of unacceptably severe side effects. Despite extensive analysis of the function of each enzyme, we sorely lack knowledge of the cell type-specific biochemical interplay between pathways. An article first published online [1] describes how Shp2 (SH2-containing tyrosine phosphatase) and Pten (phosphatase and tensin homolog), two critical regulators of central signaling pathways [2,3], have antagonistic and cooperative effects in myeloproliferation and erythropoiesis, respectively (Fig. 1). These inter-pathway studies do not always have predictable results and clearly indicate that an understanding of pathway intertwinement in major biological mechanisms is necessary to achieve better therapeutic effects.

Pten is a well-studied tumor suppressor known to inhibit the PI3K/AKT/mTOR pathway in regulation of cell growth, metabolism, and apoptosis. Pten mutations or deficiencies are

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frequently detected in various types of cancer, including acute myeloid leukemia [4]. Targeted Pten deletion in the hematopoietic compartment was found to cause myeloproliferative neoplasm as well as excessive proliferation of short-term hematopoietic stem cells, but overall decline of later-stage erythrocytes. Ptpn11/Shp2 is a proto-oncogene known to promote hematopoiesis and is implicated in juvenile myelomonocytic leukemia and other diseases [5,6]. The apparently opposite roles of Shp2 and Pten in the hematopoietic system promoted the generation and characterization of a new mutant mouse line, in which both Shp2 and Pten are deleted conditionally in hematopoietic cells.

In that study [1], phenotypic analysis was conducted through Mx1-Cre conditional knockout mice for Shp2 (SKO), Pten (PKO), and both Shp2 and Pten (DKO) to investigate gene deficiencies in the hematopoietic system. Although SKO and PKO mice had respectively decreased and increased myeloid cell populations, the DKO cell numbers were comparable to those of wild-type mice. By some means, Shp2 knockout removed Pten loss-induced extramedullary hematopoiesis and myeloid cell infiltration as well as rescued abnormal phenotypes in both the spleen and liver. Further investigation into myelopoiesis in DKO mice revealed that Shp2 deficiency rescued granulocyte and megakaryocyte progenitor, but not myeloid progenitor or LSK populations and proliferation potential. Bone marrow engraftment assays revealed that DKO cell recipients had much lower incidences of myeloproliferative neoplasm (MPN) and survived longer than PKO cell recipients in the presence of normal competitor stem cells. These data suggest that Shp2 ablation rescues part of the Pten loss-induced myeloid progenitor expansion, and the finding was somehow anticipated based on previous data on the roles of Shp2 and Pten in the blood, where Pten normally suppresses myeloproliferative neoplasm and Shp2-activating mutations are positively involved in leukemogenesis. The antagonistic relationship between Shp2 and Pten in myeloproliferation is likely a result of functional interactions between downstream effectors in the Erk and PI3K pathways, although the detailed molecular mechanisms remain to be elucidated. This is evidently not a trivial task because of the relatively low blood cell numbers available for biochemical assays, and could be even further complicated by the high level of heterogeneity of cell types in various hematopoietic lineages at different developmental stages.

The erythrocyte population came into focus with the development of lethal anemia in DKO mice that had an even shorter life span than PKO animals. Despite markedly increased erythropoietin and stable vitamin B_{12} and folic acid levels, the DKO mice had very few mature red blood cells. The phenotype was a result of shifting cell populations, with early progenitor counts higher than those of either SKO or PKO, but showing critically reduced late-stage erythrocyte counts. This analysis suggests that Shp2-or Pten-modulated signals were drastically cooperating—either directly or indirectly throughout the various differentiation stages—to maintain a stable pool of early-stage progenitors and to protect late-stage maturation of erythrocytes.

The mechanism of erythrocyte loss was attributed to cell-autonomous, rather than environmentally induced, reasons. Shp2 knockout-aggravated Pten loss induced high ROS levels of reactive oxygen species. As additional Shp2 deletion slightly reduced the dramatically elevated phospho-Akt signal induced by Pten removal, the higher level of

reactive oxygen species in DKO erythrocytes was attributed to changes in multiple pathways. Although antioxidants restored some red blood cell and hematocrit values, this partial rescue of the anemia phenotype suggests other mechanisms are needed to explain the full defects in erythrocyte differentiation and survival. This conclusion is not surprising, given the multiple Shp2 and Pten functions in hematopoietic cell development and activity. However, the cell type-specific cooperative and antagonistic effects within the same system redefine our understanding of the predictability of biochemical interactions, especially with biological systems consisting of multiple interrelated cell types. Independently analyzed signaling roles cannot be depended on in systems-level analysis of more than one molecule.

Finally, to determine the significance or translational value of the mouse genetic data in therapeutics, the authors used a pharmacologic chemical to inhibit Shp2 in wild-type and PKO mice. Of note, use of the Shp2 inhibitor revealed the therapeutic value of the compound in leukemia induced by Pten deficiency. Although the best possible effect was not explored by careful research on optimal drug dosages, the data did provide a proof of principle for treatment of leukemia patients with loss-of-function Pten mutations or dominant mutations that activate the PI3K/AKT pathway. Consistently, transplantation of PKO or DKO cells, together with normal competitor stem cells, suggested a possible beneficial effect of abrogating malignant clones, with recovery of healthy hematopoietic clones. However, the injection of Shp2 inhibitor also reproduced the anemia phenotype in PKO animals, though not as severely as in DKO mice. This observation reinforces the concept that combined Shp2 and Pten deficiency causes severe anemia. Because Shp2 promotes the Ras–Raf–Mek–Erk pathway, the authors also tested trametinib, a Mek inhibitor recently approved for treatment of patients with cancer, including pancreatic cancer and melanoma, to identify therapeutic application of the gene ablation data. Indeed, the Mek inhibitor induced anemia in Pten-deficient, but not in wild-type mice, implicating at least one molecular mechanism of the frequently seen side effect in cancer patients treated with the compound. Therefore, it will be important to perform genetic screening of blood cells for potential Pten mutations or expression deficiency, as a result of genetic or epigenetic reasons, in cancer patients prescribed pharmaceuticals, such as trametinib, in the clinic. Data from these screenings will guide physicians to predict potential side effects and make patient-specific adjustments of drug selections and dosages.

This article both uncovers a novel understanding of Pten and Shp2 properties in the blood system and backs a liberal way of thinking, through phenotypic characterization of a compound mutant mouse line in combination with mechanistic and pharmacologic studies. Viewing signaling crosstalk in this manner helps us to elucidate the effects of biochemical interactions between intra-signaling pathways and across a temporal system of differentiating cell types. As we advance in our biochemical map of interactions, these inter-pathway studies should become a major focus in therapeutic research as well as a new constant to test for in novel pathologic findings. Defining pathway interactions in various cellular systems advances therapeutic efforts by refining patient-targeted therapeutics and helping researchers redefine how distinct responses to pharmaceuticals may arise.

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References

1. Zhu HH, Luo X, Zhang K, et al. Shp2 and Pten have antagonistic roles in myeloproliferation but cooperate to promote erythropoiesis in mammals. Proc Natl Acad Sci U S A. 2015; 112:13342–13347. [PubMed: 26460004]
2. Chan RJ, Feng GS. PTPN11 is the first identified proto-oncogene that encodes a tyrosine phosphatase. Blood. 2007; 109:862–867. [PubMed: 17053061]
3. Song MS, Salmena L, Pandolfi PP. The functions and regulation of the PTEN tumour suppressor. Nat Rev Mol Cell Biol. 2012; 13:283–296. [PubMed: 22473468]
4. Worby CA, Dixon JE. Pten. Annu Rev Biochem. 2014; 83:641–669. [PubMed: 24905788]
5. Tartaglia M, Niemayer CM, Fragale A, et al. Somatic mutations in PTPN11 in juvenile myelomonocytic leukemia, myelodysplastic syndromes and acute myeloid leukemia. Nat Genet. 2003; 34:148–150. [PubMed: 12717436]
6. Loh ML, Vattikuti S, Schubbert S, et al. Mutations in PTPN11 implicate the SHP-2 phosphatase in leukemogenesis. Blood. 2004; 103:2325–2331. [PubMed: 14644997]

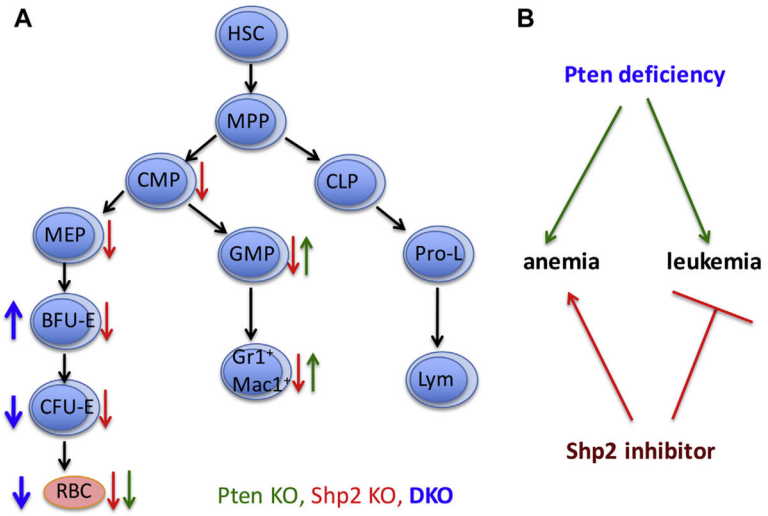

Figure 1.

Shp2 and Pten in leukemia and anemia. (**A**) Simplified hematopoietic lineage tree marked with the effects of targeted gene deletion. The *red and green arrows* indicate increases and decreases in the cell populations in Shp2 KO (SKO) and Pten KO (PKO), respectively. Ablation of Shp2 compromised the effect of Pten deletion in myelopoiesis. The *blue arrow* specifically denotes the combined effect of dual Shp2 and Pten knockout in DKO mice. The increase in BFU-E was followed by a dramatic decrease in CFU-E in DKO mice, which along with elevated reactive oxygen species, impaired survival, and other defects, eventually leads to severe anemia. (**B**) Pten deficiency promotes leukemia development and also causes mild anemia. Shp2 inhibitor can suppress the leukemogenic effect of Pten loss, but may trigger severe anemia in the background of Pten deficiency. This calls for caution and genetic screening for Pten mutations or deficiency in cancer patients before prescription of pharmaceuticals designed to suppress the Ras–Erk pathway. *BFU-E* = Erythroid burst-forming units; *CFU-E* = erythroid colony-forming units; *MPP* = multipotent progenitors; *CMP* = common myeloid progenitors; *CLP* = common lymphoid progenitors; *MEP* = megakaryocyte/erythrocyte progenitors; *GMP* = granulocyte/macrophage progenitors; *Pro-L* = prolymphocytes; *Lym* = lymphocytes; *RBC* = red blood cells.