PTEN in Regulating Hematopoiesis and Leukemogenesis

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PTEN is one of the most frequently mutated tumor suppressor genes in human cancers. By counteracting the PI3K/AKT/mTOR pathway, PTEN plays an essential role in regulating hematopoietic stem cells (HSCs) self-renewal, migration, lineage commitment, and differentiation. PTEN also plays important roles in suppressing leukemogenesis, especially T-cell acute lymphoblastic leukemia (T-ALL). Herein, we will review the function of PTEN in regulating hematopoiesis and leukemogenesis and discuss potential therapeutic approaches against leukemia with *PTEN* mutations.

P^*TEN* (phosphatase and tensin homolog deleted on chromosome 10, also named MMAC1 and TEP1), was identified as a tumor suppressor gene by three independent groups in 1997 (Li and Sun 1997; Li et al. 1997; Steck et al. 1997). As the second most frequently mutated tumor suppressor gene, *PTEN* is located on chromosome 10q23, a hotspot for deletions and mutations found in 15 or more human cancer types (Bailey et al. 2018). Germline mutations of *PTEN* are associated with hereditary cancer predisposition syndromes, such as Cowden disease and Bannayan–Zonana syndrome, with an elevated incidence of breast and thyroid cancers (Liaw et al. 1997; Nelen et al. 1997).

The protein product of *PTEN* possesses both lipid and protein phosphatase activities, as well as phosphatase-independent activities (Worby and Dixon 2014). The most well-studied PTEN functions rely on its lipid phosphatase activity by counteracting the PI3K/AKT/mTOR signaling pathway. PTEN regulates a plethora of cellular functions, ranging from stem cell self-renewal and genomic integrity to cell proliferation, survival, metabolism, and migration (for review, see Stiles et al. 2004; Song et al. 2012). Over the past 20 years, various genetic and genomic approaches have led to a comprehensive view on the roles of PTEN in regulating hematopoiesis and leukemia/lymphoma development.

Hematopoiesis is vital for all mammals; it plays essential roles in transporting nutrients, removing wastes, and preventing infections. Hematopoiesis produces billions of mature blood cells of multilineages daily to maintain blood circulation and homeostasis. In responding to infections, emergencies as well as hematopoietic

malignancies, increasing in a specific lineage(s) of blood cells are often observed (Wilson et al. 2008; Essers et al. 2009; Baldridge et al. 2010; Takizawa et al. 2012).

The hematopoietic stem cells (HSCs) are the common ancestor of all blood cell types. HSCs can self-renew and differentiate into all blood lineages (Weissman 2000). Although the concept of HSCs was developed in the late 19th century, the existence of HSCs, with the capacity of reconstituting all blood lineages in lethally irradiated recipients, was experimentally demonstrated much later (Till and McCulloch 1961; Becker et al. 1963). In the following decades, a hierarchy map was developed, illustrating a stepwise differentiation process from HSCs to mature blood cells. The long-term HSC (LT-HSC) resides at the top of the hierarchy (Smith et al. 1991; Osawa et al. 1996; Christensen and Weissman 2001), which can transit to short-term HSCs (ST-HSCs) with lower self-renewal capacity (Morrison et al. 1997; Yang et al. 2005). Multilineage progenitor cells are generated after losing some lineage potentials, such as lymphoid-primed multipotent progenitors (LMPPs) that preferentially produce lymphoid lineages, followed by single lineage-specific progenitors, such as CFU-E for red blood cell lineage (Adolfsson et al. 2001, 2005; Forsberg et al. 2006; Boyer et al. 2011). As summarized in this review, PTEN plays an essential role in regulating self-renewal, migration, lineage commitment, and differentiation of HSCs.

Related to the essential roles of PTEN in regulating HSCs and hematopoiesis, *PTEN* mutations have been found in human leukemias and lymphomas, especially in T-cell acute lymphoblastic leukemia (T-ALL). The availability of small-molecule inhibitors against the PTEN-controlled PI3K–AKT–mTOR pathway has provided new therapeutic opportunities for treating those leukemias and lymphomas with *PTEN* mutations.

PTEN IN REGULATING HEMATOPOIESIS

Most of our knowledge on the roles of PTEN in regulating hematopoiesis comes from genetically engineered mouse models, of which the murine homolog *Pten* is deleted at a specific hematopoietic stage or in a specific lineage using the Cre–Loxp system. Table 1 summarizes the published *Pten* hematopoietic-specific deleted lines and their associated phenotypes.

PTEN Controls HSC Self-Renewal

The role of PTEN in negatively regulating somatic stem cell proliferation and survival was first reported when *Pten* was deleted in the neural stem cells in vivo (Groszer et al. 2001). Subsequent analyses using in vitro neurosphere culture further demonstrated that PTEN negatively regulates neural stem cell self-renewal by modulating its G_0–G_1 cell-cycle entry and growth factor dependency (Groszer et al. 2006). Although these findings are interesting and important, the in vivo proof for the role of PTEN in regulating stem cell self-renewal is lacking because of the limitations of the neural stem cell system.

HSC is the most well-established somatic stem cell system where stem cell self-renewal can be easily and more definitively studied in vivo (Weissman et al. 2001). pIpC-inducible Mx-1-Cre line was first used to study the role of PTEN in adult HSCs (Yilmaz et al. 2006; Zhang et al. 2006). PTEN loss indeed drives quiescent LT-HSCs entering cell cycle, leaving the bone marrow niche and accumulating in the spleen. *Pten*-null HSCs in this model lose their ability for long-term reconstitution after transplantation and are eventually depleted. The above-mentioned phenotypes can be rescued by rapamycin treatment, suggesting that constitutive mTOR activation has a detrimental effect on HSC stemness maintenance (Yilmaz et al. 2006). Mechanistically, mTOR activation can induce aberrantly high rates of protein synthesis, which contributes to impaired *Pten*-null HSC self-renewal (Signer et al. 2014).

The FOXO family of transcription factors, which are downstream effectors of the PI3K-AKT pathway, are known to be important for maintaining the HSC pool by eliminating reactive oxygen species (ROS) (Tothova et al. 2007). However, *Pten*-null HSC depletion is not due to ROS, but rather by mTOR-regulated p53

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Table 1. *Pten* deletion in hematopoietic lineages and associated phenotypes

Cell type	Cre line	Phenotype	References
HSCs			
Fetal liver HSCs	VE-Cadherin-Cre	MPD followed by T-ALL; impaired B-cell development; $Tcr\alpha/\delta$-c-myc translocation and β-catenin activation; T-ALL is initiated by Lin^-CD3^+c-$Kit^{mid}Tim$-3^{high} LSCs; escape RAG-mediated β-selection	Lesche et al. 2002; Guo et al. 2008, 2011; Schubert et al. 2014; Zhu et al. 2018
	Vav-iCre	MPD followed by T-ALL	Tang et al. 2013
	CD45:Cre	Development of T-ALL but no other types of hematopoietic malignancies	Mirantes et al. 2016
Adult HSCs	Mx-1-Cre	MPD followed by transplantable AML/ALL; B lineage defect; increased HSC G_0–G_1 cell-cycle entry, mobilization, and relocation to the spleen; HSC exhaustion and impaired reconstitution ability	Yilmaz et al. 2006; Zhang et al. 2006; Tesio et al. 2013
	Scl-CreER^T	MPD followed by transplantable T-ALL; HSCs are mobilized and relocated to the spleen	Tesio et al. 2013
T cells			
DN T cells	Lck-Cre	$CD4^+$ T-cell lymphomas; defect in negative selection; altered central and peripheral tolerance; bypass IL-7 and pre-TCR-mediated signaling; elevated B cells and hypergammaglobulinemia	Suzuki et al. 2001; Hagenbeek et al. 2004; Xue et al. 2008
$CD4^+$ T cells	CD4-Cre	DP T-cell lymphoma; $Tcr\alpha/\delta$-c-myc translocation; Notch mutation	Hagenbeek and Spits 2008; Liu et al. 2010; Newton et al. 2015
B cells			
Pro-B cells	CD19-Cre	Decreased follicular B cells; enhanced migration; preferred marginal zone B cell and B1 cell generation; hyperproliferation; defect in immunoglobulin CSR and impaired AID induction	Anzelon et al. 2003; Suzuki et al. 2003
Pre-B ALL cells	Cre-ER^{T2}	Trigger central B-cell tolerance checkpoint and cell death	Shojaee et al. 2016
Myeloid cells	LysM-cre	Augmented neutrophil *trans*-endothelial migration during inflammation; Defective clearance of intracellular parasites in macrophage	Zhu et al. 2006; Sarraj et al. 2009; Kuroda et al. 2008

(HSCs) hematopoietic stem cells, (MPD) myeloproliferative disorder, (T-ALL) T-cell acute lymphoblastic leukemia, (LSC) leukemia stem cell, (AML) acute myeloid leukemia, (DN T) CD4/CD8 double-negative T cells, (ALL) acute lymphoblastic leukemia, (DP) double-positive, (AID) activation-induced cytidine deaminase.

and p16^{Ink4a} expression. Deletion of p16^{Ink4a}, p16^{Ink4a}/p19^{Arf}, or p53 (but not p19^{Arf}) can rescue the reconstitution capacity of *Pten*-null HSCs (Lee et al. 2010). mTOR exists in two distinct functional complexes, mTORC1 and mTORC2. mTORC1 controls cell proliferation and growth by activating downstream S6 kinase and mTORC2 directly phosphorylates and thereby activates AKT (Guertin and Sabatini 2007). Further genetic studies show that both mTORC1 and mTORC2 play nonredundant roles in phenotypes associated with *Pten* deletion (Kalaitzidis et al. 2012; Magee et al. 2012). In addition, inactivation of mTORC2 abrogates *Pten*-null HSCs phenotype in adult, but not in neonatal mice, indicating that PTEN/mTORC2 signaling axis functions in HSCs in a temporally dependent manner (Magee et al. 2012).

The Mx-1-Cre is a very powerful tool for pIpC-inducible gene deletion. However, Mx-1-Cre is not only expressed in HSCs but also in other hematopoietic cells and tissues (www.jax .org/strain/002527). PTEN loss in those cell types may send a paracrine signal to HSCs, complicating the phenotype analysis. pIpC-induced interferon (IFN)-α can also promote dormant HSCs to enter the cell cycle (Essers et al. 2009), which may synergize with the effect of PTEN loss on HSCs. To circumvent these issues, a fetal liver HSC specifically expressed VE-Cadherin-Cre (VE-Cad-Cre) line was used (Guo et al. 2008). Fetal liver *Pten* deletion does not cause HSC exhaustion in adults (unpubl.). Similarly, tamoxifen (Tx)-inducible Scl-CreERT (Scl-Cre)-mediated *Pten* deletion in adult HSCs shows no HSC exhaustion either (Tesio et al. 2013).

The differences seen in Mx-1-Cre versus VE-Cad-Cre or Scl-Cre-mediated *Pten* HSC deletions could be a result of the nature of Cre lines used or the impacts of genetic background (Freeman et al. 2006). Besides IFN-α induced HSC activation, high levels of G-CSF secreted by *Pten*-deficient myeloid cells can mobilize HSCs from the bone marrow to the spleen, suggesting that a non-cell-autonomous mechanism may contribute to HSC mobilization in *Pten*-null models (Tesio et al. 2013). Yet other studies argue that PTEN-modulated HSC cell–cell con-

tacts or response to inflammatory cytokines plays more important roles in HSC mobility (Li et al. 2016; Porter et al. 2016).

PTEN Controls HSC Lineage Commitment and Functions

Pten deletion in both fetal liver and adult HSCs leads to increased myeloid and T lineages, accompanied by decreased common lymphoid progenitors (CLPs) and arrested B-cell development (Zhang et al. 2006; Guo et al. 2008). Competitive reconstitution assay also prove that *Pten*-deficient HSCs have altered lineage potential. However, it is not clear whether the alterations seen in the T, B, and myeloid lineages are separate events or the result of an intrinsically linked control mechanism. A recent study demonstrates that PTEN loss in the HSC can lead to up-regulated *Spi1* transcription factor expression in early T progenitors (ETPs), before T-cell commitment (Zhu et al. 2018). Because SPI1 plays an essential role in regulating HSCs and different hematopoietic lineages (Rothenberg et al. 2016), it may serve as the central node for PTEN-regulated lineage commitment.

T-Cell Lineage

The T lineage commitment starts from ETPs in the bone marrow, and then migrates into the thymic cortex. Within the thymus, multistep T-cell proliferation and differentiation occur temporally and spatially, including T-cell commitment, *Tcrβ* locus rearrangement, β-selection, *Tcrα* locus rearrangement, and negative/positive selections. PTEN plays essential roles in almost every step of T-cell development and PTEN loss leads to T-cell lymphoma, T-ALL, and T-cell autoreactivity.

Pten deletion in HSCs leads to T-ALL development with 100% penetration (Guo et al. 2008; Tesio et al. 2013). In the *VE-Cad-Cre; Pten$^{L/L}$* model, PTEN loss leads to premature *Tcrβ* locus rearrangement and enables *Rag1$^{-/-}$* T cells bypassing β-selection to differentiate to double-positive (DP) cells (Guo et al. 2011; Zhu et al. 2018). PTEN loss or PI3K activation provides sufficient nutrients to meet the needs of T-cell

differentiation from the double-negative (DN) to DP stage and mTOR serves as a critical signaling mediator for this process (Guo et al. 2011). Similarly, *Pten* deletion in DN (Lck-Cre) or CD4^{+} T cells (CD4-Cre) leads to DP T-cell lymphomas, and T-cell development in these models can also bypass RAG-mediated β-selection as well as the requirements of IL-7 and pre-TCR-mediated signaling (Hagenbeek et al. 2004; Hagenbeek and Spits 2008; Liu et al. 2010). These results suggest that constitutive activation of the PI3K/AKT/mTOR pathway can send an "inside-out" signal and promote T-cell differentiation in the absence of the "outside-in" signaling from the pre-TCR complex.

Pten heterozygous mice with one allele of *Pten* T-cell-specific deleted show spontaneous activation of CD4^{+} T cells, defects in negative selection during development, as well as central and peripheral tolerance, suggesting that PTEN is an important regulator for T-cell homeostasis and self-tolerance (Suzuki et al. 2001). Interestingly, this model also shows increased B cells and hypergammaglobulinemia, a phenotype not reported in other T-cell-specific *Pten* deletion models (Table 1), suggesting that this phenotype may be contributed by the *Pten* heterozygous environment rather than *Pten*-deficient T cells.

B-Cell Lineage

Similar to T-cell, B-cell development also undergoes a multistep process, including prepro-B, pro-B, pre-B, immature B, and B cells with several checkpoints (for review, see Melchers 2015). *Pten* deletion in both fetal liver and adult HSCs leads to decreased CLPs, arrested B-cell development after prepro-B progenitor stage, and lost white pulp in the spleen (Zhang et al. 2006; Guo et al. 2008). The mechanism underlying PTEN-controlled B-lineage commitment and differentiation is currently unknown but blocking leukemia stem cell (LSC) maintenance and T-ALL development in the *VE-Cad-Cre; Pten^{L/L}* model can restore white pulp in the spleen, suggesting a potential link between prepro-B progenitors and T-ALL LSC formation and function (Zhu et al. 2018).

CD19 is a cell-surface protein expressed specifically in B lineage from pro-B to mature B stages. CD19-Cre-mediated *Pten* deletion can substitute the function of CD19 and rescue B1 and marginal zone B-cell formation in *Cd19^{-/-}* mice (Anzelon et al. 2003). *Pten*-deficient B cells are hyperproliferative and resistant to apoptotic stimuli. Immunoglobulin class switch recombination is defective, suggesting that PTEN plays an important role in B-cell development and homeostasis (Suzuki et al. 2003).

Studies on the role of PTEN in pre-B-ALL suggest that PTEN is a critical gatekeeper of B-cell tolerance, which is known to be controlled by an intermediate level of PI3K signaling. PTEN loss in pre-B-ALL leads to cell death (Shojaee et al. 2016). PI3K also controls the activities of the FOXO family of transcription factors. Selective *Foxo1* deletion at different B-cell developmental stages leads to early B-cell development blockade (Suzuki et al. 2003; Dengler et al. 2008). These results collectively indicated that PTEN is essential for B-cell homeostasis, including B-cell commitment, development, and maturation following stimulation.

Myeloid Lineage

Pten deletion in both the fetal liver and adult HSCs leads to myeloproliferative disorder (MPD) (Zhang et al. 2006; Guo et al. 2008). However, it is not clear whether MPD is the precursor of acute myeloid leukemia (AML) observed in the Mx-1-Cre-mediated *Pten* deletion model (Yilmaz et al. 2006) because MPD phenotypes are present in VE-Cad-Cre and Scl-Cre *Pten* models without AML development (Guo et al. 2008; Tesio et al. 2013).

The PTEN-C/EBPA-CTNNA1 signaling axis, which is evolutionarily conserved in humans, mice, and zebrafish, may contribute to the expansion of myeloid lineage and transformation of HSCs and myeloid progenitors (Fu et al. 2010). *Pten* deletion causes up-regulation of the PI3K pathway in the myeloid cells, which secrete high levels of G-CSF and stimulate myeloid progenitor cell proliferation (Tesio et al. 2013). Furthermore, mice with macrophage *Pten* deletion have reduced the ability to elimi-

nate parasites (Kuroda et al. 2008) while *Pten*-deleted neutrophils have enhanced invasive ability and can be readily recruited to inflamed sites (Sarraj et al. 2009).

PTEN IN REGULATING LEUKEMOGENESIS

PTEN Deletion and Mutations in Human Leukemia and Lymphomas

PTEN is functionally lost in a range of human cancers through mutation, deletion, transcriptional silencing, and protein inactivation (Salmena et al. 2008). *PTEN* mutation or deletion have been found in hematological malignancies but with much lower frequencies, as compared to that of solid tumors (Hollander et al. 2011). Among all hematopoietic malignancies, T-ALL has the highest rates of *PTEN* deletion or mutation, while few or no *PTEN* deletion/mutations have been reported in AML and B-ALL, respectively (see Fig. 1). *PTEN* deletions or mutations

are also found in T-cell lymphomas and large B-cell lymphomas (Fig. 1).

The high *PTEN* mutation frequency in T-ALL and T-cell lymphomas is probably related to the essential role of PTEN or PI3K pathways in T-cell lineage commitment and differentiation, as discussed in the above section. The reason for low mutation rate in AML is currently unknown but may relate to increased SPI1 levels in the PTEN-null early progenitors (unpubl.) as *Spi1* knockout or decreased expression is known to associate with AML (Verbiest et al. 2015). B-cell tolerance is controlled by an intermediate level of PI3K signaling. PTEN loss in pre-B-ALL leads to hyperactivated PI3K signaling and consequently cell death, which may explain the undetectable PTEN mutations in B-ALL (Shojaee et al. 2016). On the other hand, a large-scale study of diffuse large B-cell lymphoma (DLBCL) demonstrates that PTEN dysregulation at multiple levels correlates to poor prognosis of DLBCL patients (Wang et al. 2018), suggesting PTEN loss
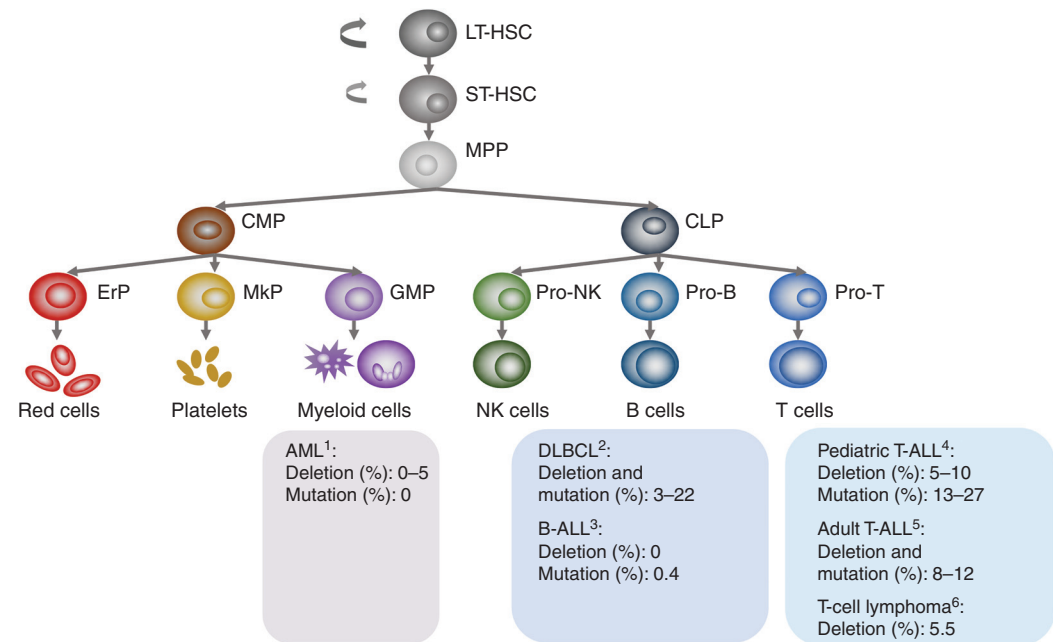
Figure 1. PTEN alterations in human leukemias and lymphomas. (AML) acute myeloid leukemia, (B-ALL) B-cell acute lymphoblastic leukemia, (DLBCL) diffuse large B-cell lymphoma, (T-ALL) T-cell acute lymphoblastic leukemia. [1]Yoshida et al. (2011); Cancer Genome Atlas Research Network et al. (2013); Ma et al. (2018). [2]Reddy et al. (2017); Wang et al. (2018). [3]Ma et al. (2018). [4]Gutierrez et al. (2009); Jenkinson et al. (2016); Liu et al. (2017); Ma et al. (2018). [5]Trinquand et al. (2013); Neumann et al. (2014). [6]Sondka et al. (2018).

at different stages of B-cell development may result in different consequences.

The frequencies of *PTEN* mutation in T-ALL vary significantly in the earlier literature. Recent large-scale analyses revealed that 14% of pediatric and young adults (Liu et al. 2017) and 19% of pediatric T-ALL samples carried *PTEN* mutations (Seki et al. 2017). When combining data from all major papers, the frequency of *PTEN* mutations ranges from 8% to 12% in adults (Trinquand et al. 2013; Neumann et al. 2014) and 13% to 27% in pediatric T-ALLs (Fig. 1; Gutierrez et al. 2009; Zuurbier et al. 2012; Balbach et al. 2016; Jenkinson et al. 2016; Liu et al. 2017; Seki et al. 2017).

PTEN somatic mutations in T-ALL are concentrated in exon 7, different from exon 5 found in solid cancers (Fig. 2; Zuurbier et al. 2012; Jenkinson et al. 2016; Tesio et al. 2016; Liu et al. 2017). The exon 5 encodes the phosphatase domain and most mutations in the exon 5 lack phosphatase activity. The exon 7, on the other hand, encodes part of the C2-domain, which is important for lipid membrane binding, p53, and cell-migration regulations (Georgescu et al. 2000; Freeman et al. 2003; Raftopoulou et al. 2004), suggesting that a phosphatase-independent mechanism may be involved in PTEN-regulated leukemogenesis.

Besides genomic alterations, transcription, posttranscription, and posttranslation mechanisms can also lead to PTEN loss of function (Salmena et al. 2008). Alterations in the components of PI3K/AKT signaling pathway are also observed in T-ALL, especially in the pediatric T-ALL. Approximately 29%–88% of human pediatric T-ALL patients have hyperactivated PI3K/AKT pathways, which could be the result of *PTEN*, *PIK3R1*, and *AKT1* mutations (Silva et al. 2008; Gutierrez et al. 2009; Liu et al. 2017).

Pten-Null Mouse Models for Human Leukemia and Lymphomas

To understand the molecular and cellular mechanisms underlying PTEN-regulated leukemia and lymphoma development, a series of mouse models have been generated. Similar to high frequent *PTEN* mutations found in human T-ALL

and T-cell lymphomas, the most prevalent phenotypes associated with *Pten* HSC and hematopoietic lineage–specific deletions are T-ALL and T-cell lymphomas (Table 1; Fig. 1).

VE-Cad-Cre-mediated *Pten* deletion in fetal liver HSCs leads to T-ALL but not leukemia in other lineages (Guo et al. 2008, 2011). PTEN loss, β-catenin activation, and *Tcrα/δ-c-Myc* translocation-mediated T-cell-specific *Myc* overexpression are three essential events, which are also found in human T-ALLs (Guo et al. 2008, 2011; Schubbert et al. 2014). Similarly, *Vav-iCre*-, *Scl-Cre*-, and *Cd45-Cre*-mediated *Pten* deletions in HSCs and CD45$^+$ cells also lead to T-ALL development in adult mice without causing other types of leukemia (Tang et al. 2013; Tesio et al. 2013; Mirantes et al. 2016). pIpC-induced and Mx1-Cre-mediated *Pten* adult HSC deletion can cause both AML and T-ALL (Yilmaz et al. 2006; Zhang et al. 2006), which may be the result of pIpC-induced HSC activation or PTEN loss in non-HSC cells, as described in the previous section. Reactivation of PTEN via a tetracyclin-dependent system can reduce T-ALL dissemination but not leukemic burden in hematopoietic organs, suggesting that the role of PTEN in T-ALL may be dictated by the tissue microenvironment (Miething et al. 2014).

Pten deletion at different stages of T-cell development can also lead to T-cell lymphoma development (Table 1). Interestingly, *Tcrα/δ-c-Myc* translocation has also been observed in *Pten*-deficient T-cell lymphomas (Liu et al. 2010).

PTEN Controls Leukemia Stem Cells

Cancer stem cells (CSCs) are a rare subpopulation found in various cancers, including hematopoietic malignancies (Batlle and Clevers 2017). CSCs were first identified in AML patients (Bonnet and Dick 1997), and were named leukemia-initiating cells (LICs) or LSCs. Analog to normal HSCs, LSCs have the ability of self-renewal and differentiation (Reya et al. 2001). However, there are very few publications on the identity of human T-ALL LSCs (Cox et al. 2007; Chiu et al. 2010).
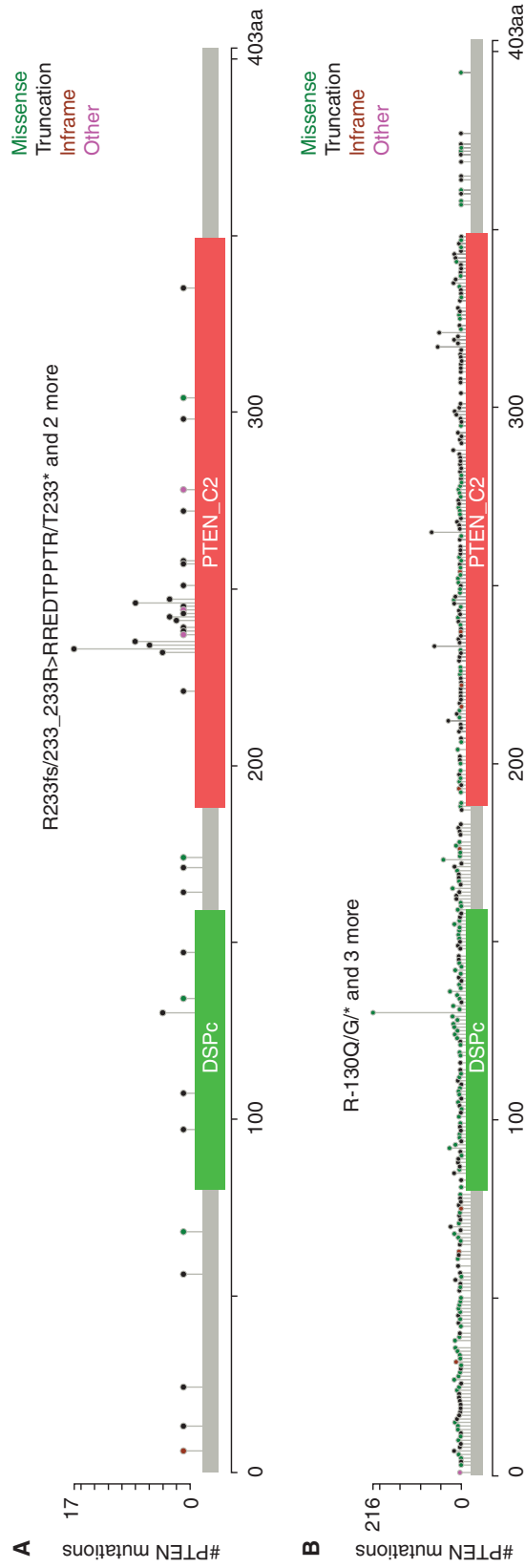
Figure 2. PTEN somatic mutations in human T-cell acute lymphoblastic leukemia (T-ALL) and solid tumors. (*A*) PTEN somatic mutations found in T-ALL. Data collected from Liu et al. (2017) and Seki et al. (2017) along with our unpublished results. (*B*) PTEN somatic mutations found in solid tumors. Data adapted from cBioPortal for Cancer Genomics. (DSPc) dual specificity phosphatase, catalytic domain (aa80–159), (PTEN_C2) C2 domain of PTEN tumor-suppressor protein (aa188–349).

LSCs have been identified within the Lin⁻CD3⁺c-kit^mid HAVCR2^high subpopulation in the *VE-Cad-Cre; Pten^{L/L}* model (Guo et al. 2008; Zhu et al. 2018). *Pten* deletion, β-catenin activation, and *Tcrα/δ-Myc* translocation, the aforementioned three events essential for T-ALL development, are also important for LSC formation (Guo et al. 2008, 2011; Schubbert et al. 2014). Genetic deletion of *β-catenin* in the *Pten*-null T-ALL model can block LSC formation and T-ALL development (Guo et al. 2008). T-ALL initiated by β-catenin activation also has *Tcrα/δ-Myc* translocation and *Pten* deletion (Kaveri et al. 2013; Dose et al. 2014), indicating that the presence of the three essential events, rather than the order of the events, are important for LSC formation. LSC formation is recombinase-activating gene (RAG)-dependent as deletion of RAG1 in the *Pten*-null T-ALL mice can prevent *Tcrα/δ-Myc* translocation and T-ALL development (Liu et al. 2010; Guo et al. 2011). Rapamycin, a specific inhibitor of the mTOR pathway, can mimic *Rag1* deletion in suppressing LSC formation and T-ALL development at the initial stage of T-ALL development (Guo et al. 2011).

The maintenance of *Pten*-null LSC "stemness," however, requires another layer of a control mechanism, that is, by the master regulator SPI1 and the β-catenin-SPI1-HAVCR2 regulatory circuit (Zhu et al. 2018). SPI1 is an ETS domain transcription factor, which is essential for LSC-signature gene expression and LSC activity. Deletion of *Spi1* in the *Pten*-null T-ALL model can completely block LSC formation. Although initiated by PI3K-controlled β-catenin activation, the LSC-specific expression of *Spi1* is reinforced by the β-catenin-SPI1-HAVCR2 regulatory circuit. Once formed, LSCs are very sensitive to any perturbation of this regulatory circuit, either genetically or pharmacologically, but less dependent on its driver mutations, as inhibiting a PTEN-controlled PI3K pathway at the leukemia stage has little effect on LSC number and T-ALL development (Guo et al. 2011; Schubbert et al. 2014; Zhu et al. 2018). *Spi1* is silenced by DNA methylation, which leads to shut down of LSC-signature gene expressions, loss of LSC "stemness," and leukemic differen-

tiation, suggesting that LSC "stemness" maintenance is reversibly controlled by an epigenetic mechanism. Whether human T-ALL LSCs are also regulated a similar mechanism remains to be seen but translocation-mediated *SPI1* overexpression has been identified recently in high-risk pediatric T-ALL patients (Seki et al. 2017; Chen et al. 2018).

Targeting PTEN-Null Leukemias and Lymphomas

PTEN mutation or constitutive activation of its downstream PI3K/AKT/mTOR signaling pathway are common events in human T-ALL and lymphomas, suggesting PI3K could be an effective target for treating PTEN-deficient leukemias and lymphomas. The PI3K inhibitors LY294002, wortmannin and BAY1082439 (Hagenbeek et al. 2014; Zhu et al. 2018), AKT inhibitor triciribine (Evangelisti et al. 2011), and mTOR inhibitors Rapamycin and pp242 (Guo et al. 2011; Schubbert et al. 2014; Kawata et al. 2018; Zhu et al. 2018) can efficiently suppress T-ALL cell growth either in vitro in cell lines or in vivo in the mouse models. The class I PI3K is comprised of α-, β-, γ- and δ- isoforms, and each has different biological functions in human cancers (Katso et al. 2001). Both p110γ and p110δ are required for *Pten*-null T-ALL development, and inhibiting the catalytic domains of p110γ and p110δ by a novel small molecular CAL-130 can induce cell apoptosis in vitro and reduce the disease burden in vivo in a *Pten*-deficient T-ALL model (Subramaniam et al. 2012).

However, the pharmacological inhibitors of the PI3K/AKT/mTOR pathway could inhibit some but not all *PTEN*-null human T-ALL cell lines in vitro, and blasts but not LSCs in the *Pten*-null mice model in vivo (Guo et al. 2011; Schubbert et al. 2014; Zhu et al. 2018), suggesting that other survival mechanisms may contribute to the drug resistance in the PTEN-null T-ALL. Dysregulated NOTCH signaling pathway is one of the most prevalent events found in human T-ALL. The NOTCH1 signaling pathway cross talks to the PI3K/AKT pathway and loss of PTEN function makes T-ALL cell lines resistant to NOTCH1 γ-secretase inhibitors

(GSIs) (Palomero et al. 2007). Further studies demonstrated that NOTCH represses *PTEN* expression through NOTCH1-HES1-mediated mechanisms (Palomero et al. 2007). In PTEN-deficient T-ALL without NOTCH pathway mutations, the DLL4/NOTCH signal plays an independent and nonredundant role (Hagenbeek et al. 2014). Similarly, a recent study shows that focal adhesion kinase (FAK) provides a parallel pathway to the PI3K/AKT/mTOR pathway, and blocking FAK signaling makes *Pten*-null T-ALL cells sensitive to PI3K/AKT inhibitors (You et al. 2015).

CSCs are considered as the root of the cancer and are responsible for cancer propagation and therapeutic resistance (Dean et al. 2005). Although T-ALL blast cells are very sensitive to PI3K inhibitors, LSCs are resistant to such treatments because of their dependency on the β-catenin-SPI1-HAVCR2 regulatory circuit (Guo et al. 2011; Schubbert et al. 2014; Zhu et al. 2018). Perturbation of this regulatory circuit by WNT/β-catenin inhibitors BAY6060/6167, SPI1 inhibitor DB1976, or HAVCR2 antibody, in combination with PI3K/AKT inhibitors rapamycin or BAY1082439, can eradicate both LSCs and T-ALL blast cells in *Pten*-null T-ALL, suggesting that the effective treatments for PTEN-null T-ALL need to cotarget both oncogenic driver mutations and "stemness" maintenance pathways (Zhu et al. 2018).

Other therapeutic strategies are also worth exploring, including metabolic therapies and immunotherapies. The PTEN/PI3K/AKT pathway plays a key role in regulating cell metabolic pathways by promoting aerobic glycolysis and protein synthesis. Deletion of *S6k1*, a downstream target of mTORC1, can significantly delay leukemia development in the *Pten* deficiency model (Tandon et al. 2011). NOTCH activation can promote glycolysis and glutamine oxidation through PI3K and MYC activation, which induces metabolic stress and activates AMPK. AMPK is essential to balance glycolysis and mitochondria function to control T-ALL cell stress and survival, and AMPK deficiency leads to T-ALL cell death and reduced disease burden (Herranz et al. 2015; Kishton et al. 2016). The PTEN or PI3K pathway also regulates cancer metabolic reprogramming by diverging glycolytic intermediates to branching metabolic pathways, such as PPP and HBP, and PTEN-null T-ALL cell lines and in vivo mouse model are also very sensitive to the PPP or HBP inhibition (unpubl.).

CAR-T-mediated immunotherapy is very effective in treating B-ALL (June et al. 2018). As a highly heterogeneous disease with different cell-surface marker expressions, recent studies have identified differential expressions of CD5, CD7, and T-cell receptor (TCR) in different subtypes of T-ALLs (Chen et al. 2017; Gomes-Silva et al. 2017; Maciocia et al. 2017; Cooper et al. 2018). However, systematic efforts are needed to identify the immunotherapy targets specific for PTEN-deficient T-ALLs.

PROSPECTIVE

After two decades of investigations, the essential roles of PTEN in regulating HSC maintenance, lineage commitment, and leukemogenesis have been well established. However, some of the important mechanisms are still lacking and translation of the promising preclinical results to patient treatments requires a joint effort among basic and clinical scientists, as well as pharmaceutical companies. Some important research areas are listed below:

1. Study PTEN-controlled hematopoiesis and leukemogenesis under homeostatic conditions. Recent studies, based on single-cell omics and lineage tracing under steady-state conditions, have redefined the hematopoietic hierarchy. Instead of a stepwise discrete process, previously defined HSCs and progenitors, are now been recognized as heterogeneous cell populations, plastic and lacking obvious boundaries (Macaulay et al. 2016; Velten et al. 2017; Buenrostro et al. 2018; Karamitros et al. 2018). Because previous studies are largely based on transplantation of FACS sorted donor cells to irradiated immune-incompetent recipients, how PTEN regulates HSCs in its native niche and in a steady-state setting requires further investigation.

Studying the function of *Pten*-null HSCs in its native niche will also help us understand the role of PTEN in regulating HSC lineage commitment. *Pten* deletion in HSCs leads to increased myeloid and T lineages, accompanied by arrested B-cell development (Zhang et al. 2006; Guo et al. 2008). Based on the new view of a hematopoietic hierarchy, these seemingly disconnected events may be intrinsically linked in progenitor(s) that can switch among different lineages. The underlying mechanism may also shed light on certain mixed-lineage phenotypes associated with human leukemia (Gutierrez and Kentsis 2018).

2. Investigate the role of PTEN in regulating the epigenetic landscape during hematopoiesis and leukemogenesis. Despite the similarity in their gene expression patterns, each HSC clone has its distinct chromatin architecture, leading to functionally distinct lineage preference (Yu et al. 2016). The impacts of epigenetic regulations on hematopoiesis and leukemogenesis are also reflected by the identification of DNMT3A, ASXL1, TET2, and IDH1/IDH2 mutations that correlate with a predisposition to hematopoietic malignancies (González et al. 2015; Corces et al. 2016; Langstein et al. 2018). Given the roles of PTEN in controlling HSC lineage commitment and leukemogenesis, investigating whether and how PTEN regulates the hematopoietic and leukemic epigenetic landscape will be important.

3. Target the key leukemia driver transcription factors. Transcription factors play essential roles in lineage choice during hematopoiesis, and their alterations can drive hematopoietic malignancies. Transcription factors are usually regarded as undruggable targets and JQ1 is the first small molecule inhibitor that can effectively inhibit the BRD4 transcription factor (Filippakopoulos et al. 2010). BRD4 is known to drive *c-Myc* expression (Zuber et al. 2011) and JQ1 has been used to target MYC-dependent AML, ALL, and other cancer types (Delmore et al. 2011; Mertz et al. 2011; Ott et al. 2012). PTEN-null T-ALLs with *Tcrα/δ-c-Myc* translocation are also sensitive to JQ1 (Schubbert et al. 2014). DB1976 is another small-molecule inhibitor that can specifically block the interaction between SPI1 and its target DNA sequences (Munde et al. 2014; Stephens et al. 2016). DB1976 has therapeutic effects on PTEN-null T-ALL and tissue fibrosis, of which SPI1 has been identified as the master regulator (Zhu et al. 2018; Wohlfahrt et al. 2019). Therefore, developing clinical grade small molecular inhibitors that can directly target c-MYC and other cancer-driving transcription factors will open new windows for leukemia treatment.

4. Cotarget both cancer driver mutations and CSC maintenance mechanisms. The discovery of LSC maintenance mechanisms, independent of the cancer driver mutations in the *Pten*-null T-ALL model (Zhu et al. 2018), may have more general and important implications for treating cancers in which CSCs are known to play essential roles. First, targeting driver mutations or dysregulated pathways may be sufficient for debulking the tumor mass but not for eliminating CSCs unless the CSC "stemness" maintenance mechanism is simultaneously inhibited. Second, since the CSC "stemness" maintenance mechanisms are likely regulated by epigenetic mechanisms, one would predict poorer outcomes if cancers controlled by such a mechanism were treated by 5-AZ or similar agents.

Collectively, what we have learned in the past two decades will pave new ways to study the role of PTEN in the hematopoietic system and open new windows to explore novel therapeutic strategies for treating hematological malignancies caused by PTEN loss.

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