Concise Review: Balancing Stem Cell Self-Renewal and Differentiation with PLZF

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ABSTRACT

In recent years, the highly conserved promyelocytic leukemia zinc finger (PLZF, also known as ZBTB16, ZNF145) has attracted attention as a multifunctional transcription factor involved in major biological processes during development. As a transcription factor, PLZF shows tight regulation in its cell-type-specific and stage-specific expression patterns. Emerging evidence shows that PLZF regulates the balance of self-renewal and differentiation in stem cells. However, the gene regulatory network of PLZF is only beginning to be understood. In this review, we discuss the diverse functions of PLZF, in particular its role in self-renewal versus differentiation of stem cells. We also discuss the current state of knowledge on the gene regulatory network of PLZF, in conjunction with its upstream factors, post-translational modifications and binding cofactors for multiprotein complexes. This review aims to provide the reader with an in-depth understanding of the molecular mechanisms underlying PLZF and the potential applications in tissue regeneration. STEM CELLS *2016;34:277–287*

SIGNIFICANCE STATEMENT

SIGNIFICANCE STATEMENT Stem cells posses the unique ability to maintain multipotency and self-renew. Stem cell fate decisions, to self-renew or differentiate, is a key step in the therapeutic use of stem cells for regenerative medicine. In recent years, the highly conserved promyelocytic leukemia zinc finger (PLZF, also known as ZBTB16, ZNF145) has attracted attention as a multifunctional transcription factor involved in stem cell biology. PLZF is expressed in long-term HSCs (LT-HSCs), spermatogonial stem cells and neural progenitors to maintain their self-renewal. Interestingly, PLZF is also expressed during myeloid differentiation, naïve T cells differentiating into effector T cells, and osteochondral differentiation of MSCs into bone and cartilage. Our labs have substantial experience with the effects of PLZF on the osteochondral differentiation of MSCs, and we would like to propose a model for how PLZF might balance stem cell self-renewal and differentiation as a transcription factor.

INTRODUCTION

Zinc finger proteins represent the most abundant protein superfamily with extraordinarily diverse functions. The Cys_2/His_2 zinc finger is a motif of the second highest abundance in the human genome and the highest abundance in DNA-binding transcription factors. The promyelocytic leukemia zinc finger (PLZF) protein belongs to the family of Krüppel-like zinc finger proteins, which are involved in the regulation of diverse cellular processes, including cell proliferation, apoptosis, differentiation, and development [1]. PLZF was first identified as a fusion partner of the retinoic acid receptor alpha (RARα) in a chromosomal translocation t(11;17) (q23;q21) implicated in acute promyelocytic leukemia (APL) [2, 3]. The C-terminus of PLZF sequence is deleted in PLZF-RARA, and the N-terminus of PLZF sequence is deleted in RARA-PLZF. PLZF-RARA acts to suppress both RARA and PLZF functions, and RARA-PLZF acts to modulate PLZF function, thus both fusion products contribute to induce acute promyelocytic leukemia. Later studies revealed that, besides myeloid cells, PLZF is expressed in a tissue- and stage-specific fashion during spermatogenesis, neurogenesis, embryonic limb bud patterning, and T cell development [4]. PLZF also enhances osteogenesis and chondrogenesis of mesenchymal stem cells (MSCs) [5, 6]. As a zinc finger transcription factor, PLZF controls the expression of lineage-specific target genes, thereby instructing stem/progenitor cells to adopt certain cell-fate programs for self-renewal or differentiation. Here we discuss the main

functions of the PLZF transcription factor and summarize its known gene regulatory network during stem cell development and tissue regeneration.

Homology and Genetics of PLZF

PLZF is a well conserved gene, from the nematode *Caenorhabditis elegans* to humans. In human, PLZF gene contains six exons and five introns [7], its exons vary from 87 to 1358 bp, distributing over a region of approximately 120 kb [8]. PLZF exhibits complex patterns of splicing in a tissue-specific manner, and at least four isoforms are detected within exon 1 [7]. The PLZF protein includes nine Kruppel-like C_2H_2 zinc finger motifs in the C-terminus, a lesser-known RD2 domain, and a BTB (bric-a-brac, tram track, broad complex)/POZ (poxvirus, zinc finger) domain in the N-terminus [2]. The nine Kruppel-like C_2H_2 zinc fingers facilitate sequence-specific DNA binding to its target genes, which allows PLZF to function as a transcription factor [9]. The BTB/POZ domain is an evolutionarily conserved motif, which mediates protein–protein interactions and allows POZ domain proteins to participate in various different processes, including hematopoiesis, angiogenesis, neurogenesis, adipogenesis, osteoclastogenesis, and muscle differentiation [10, 11].

In *Caenorhabditis elegans, lin-31* (component of ERK) null mutants are viable and have a phenotype which ortholog of PLZF *eor-1* does not affect. In addition, the phenotype of *eor-1* mutation is similar to that of *bar-1* β-catenin mutations in genetic behavior. These data show that *eor-1* acts downstream or in parallel to the ERK kinase and the Wnt signaling-suppressor *pry-1*/Axin1. EOR-1/PLZF appears to cooperate with Hox genes to promote the expression of Ras/ERK and Wnt/β-catenin target genes during differentiation of the hypodermal P12 neuroectoblasts (neural progenitors) and vulval precursor cells (genital progenitors) in nematode development 13 [12]. In *Drosophila*, the PLZF ortholog *tramtrack* is expressed downstream of Notch signaling in the peripheral nervous system, to control the differentiation of sensory organ precursor cells (neural progenitors) into neurons or glial cells [13, 14]. Transgenic Drosophila strains expressing PLZF exhibit phenotypic changes by regulating ERK signaling in eye neural progenitors and wing appendage development [15, 16]. In mice, loss-of-function mutations in PLZF cause severe skeletal defects [17] and genital hypoplasia [18, 19]. In humans, besides the above mouse phenotypes, mental retardation is also observed with loss-of-function mutations in PLZF [20]. These observations show that PLZF plays important, evolutionarily conserved roles in metazoan tissue development.

Stem Cell Self-renewal

Stem cells posses the unique ability to self-renew and maintain multipotency. Throughout their life span, stem cells decide whether to self-renew or differentiate. The regulation of stem cell homeostasis closely depends on integrating intrinsic and extrinsic signals, such as the transcription factors. PLZF has been shown to maintain stem cell homeostasis by maintaining the balance between progenitor pools and the generation of large numbers of differentiated cells [21].

HSCs and Progenitors

PLZF is highly expressed in undifferentiated hematopoietic stem cells (HSCs) and progenitors [22, 23], and downregulated during differentiation, suggesting that PLZF is involved in stem cell maintenance and self-renewal. Forced expression of PLZF is able to immortalize HSCs and myeloid progenitors in vitro. By contrast, depletion of PLZF inhibits the MLL-fusion-induced leukemic transformation of HSCs in vitro and in vivo [24]. PLZF achieves this by repressing transcription factors involved in normal myeloid differentiation, including GFI-1, C/EBPα, and LEF-1, and inducing negative regulators DUSP6 and ID2 [21] (Fig. 1A; Table 1). These data suggest that PLZF expression may be associated with the self-renewal of myeloid progenitors and HSCs. PLZF has been identified to be involved in human acute promyelocytic leukemia (APL). APL is a myeloid subtype of hematopoietic malignancies, which is known to be associated with reciprocal chromosomal translocations involving the retinoic acid receptor alpha (RARa) gene on chromosome 17 and various partner genes on distinct chromosomes [25], including a variant translocation t(11;17) (q23; 21) in which PLZF is fused to the RARA gene on chromosome 17 forming a fusion protein [2]. Fluorescence in situ hybridization localizes the PLZF gene to 11q23.1 [3]. Molecular studies reveal that the PLZF/RARA fusion protein acts mainly as an epigenetic regulator of its target genes by directly interacting with the Polycomb protein Bmi-1 to recruit PRC1 to the retinoic acid response elements [26]. On the other hand, RARA/PLZF recruits HDAC1 to cause histone H3 deacetylation at the C/EBPα locus, leading to a decrease in the expression of C/EBPα target genes, and thus inhibiting myeloid differentiation to promote self-renewal of myeloid progenitors [27].

Neural Progenitor Cells

PLZF is also expressed in dividing progenitors and downregulated during neural differentiation [28]. In Drosophila, the PLZF ortholog *tramtrack* blocks neuronal differentiation and regulates glial development from neural progenitors [14]. Similar effects are observed in vertebrates. In chicks and mice, PLZF overexpression promotes the maintenance of neural progenitors and suppresses neurogenesis. In contrast, reduced PLZF activity by shRNA compromises neural progenitor maintenance, leading to neuronal differentiation. These data show that PLZF maintains the self-renewal of neural progenitors. Further molecular analysis shows that PLZF maintains neural progenitors by upregulating FGFR3 expression and STAT3 pathway activity (Fig. 1B). However, long-term expression of PLZF biases neural progenitors towards gliogenesis [28]. In contrast, Btbd6a, an adaptor for the Cul3 ubiquitin ligase complex, binds to PLZF to promote the translocation of PLZF from the nucleus to the cytoplasm for ubiquitination and degradation, leading to neural differentiation [29] (Fig. 1B). These data suggest that PLZF is required to maintain neural progenitors and inhibit their differentiation into neurons.

Spermatogonial Progenitor Cells

PLZF plays a crucial role in maintaining spermatogonial self-renewal [18, 19]. PLZF is a widely acknowledged biomarker of type A and B spermatogonia in zebrafish [30], coexpressed with Oct4 in undifferentiated spermatogonia [19]. Adult spermatogonial stem cells are capable of self-renewal and production of

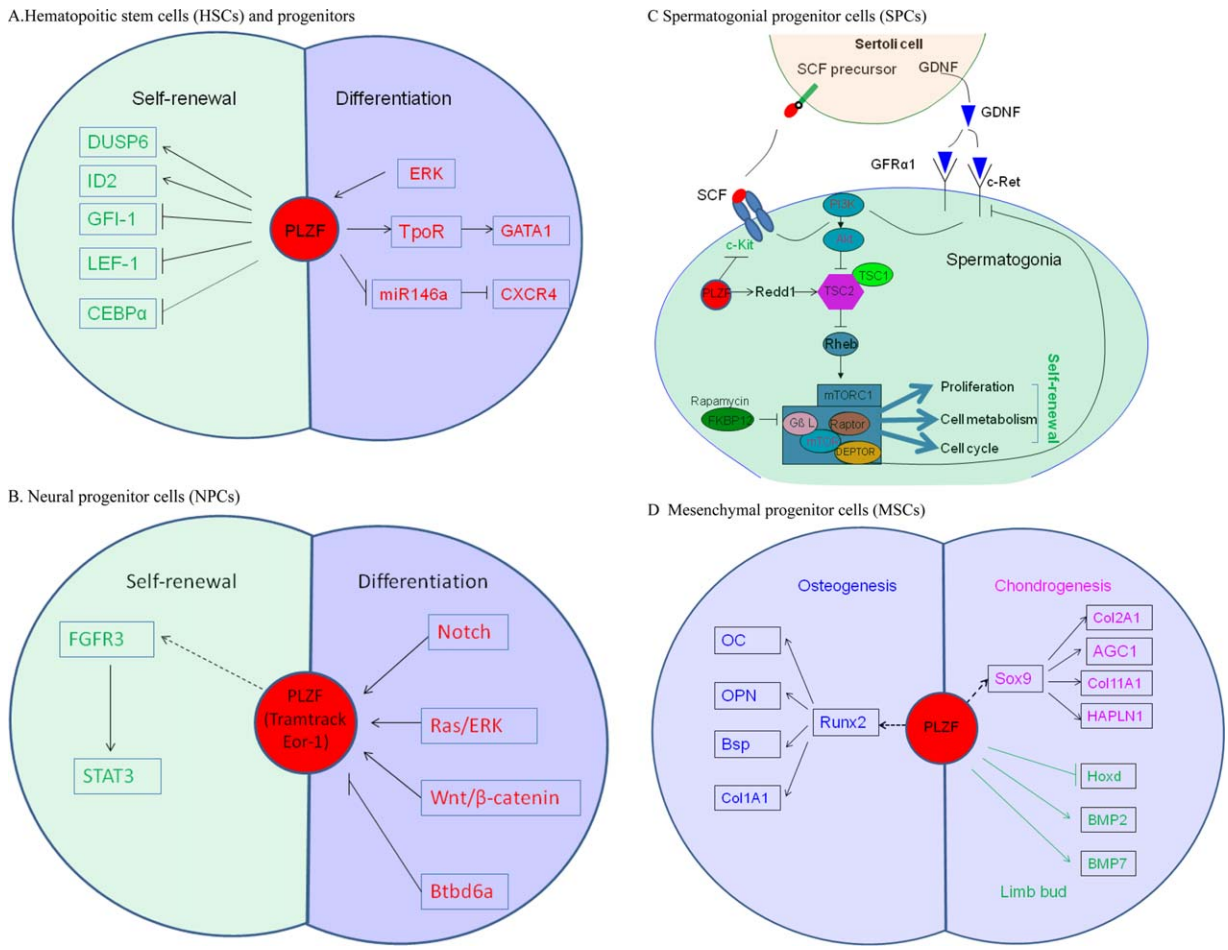

Figure 1. Regulation of balance between self-renewal and differentiation of stem cells by promyelocytic leukemia zinc finger (PLZF). **(A)**: Balance between self-renewal and differentiation of hematopoietic stem cells (HSCs) and progenitors by PLZF. PLZF maintains self-renewal of HSC and progenitor by repressing differentiation-related genes GFI-1, LEF-1, CEBPα, and inducing DUSP6 and ID2. Upon stress, ERK1/2 induced by cytokines leads to nuclear export and inactivation of PLZF, which leads to c-kit activation for myeloid differentiation. PLZF also stimulates megakaryopoiesis by inducing TpoR or suppressing miR-146a to activate CXCR4. **(B)**: Balance between self-renewal and differentiation of neural progenitor cells (NPCs) by PLZF. PLZF maintains the self-renewal of neural progenitors by upregulating FGFR3 and STAT3 pathway activity. On the contrary, eor-1/PLZF acts downstream of Ras/ERK and Wnt/β-catenin during differentiation of P12 neurectoblasts and vulval precursor cells in nematode development. PLZF ortholog tramtrack is downstream of Notch signaling to control the differentiation of sensory organ precursor cells (neural progenitors) to neurons or glial cells. In addition, Btbd6a, an adaptor for the Cul3 ubiquitin ligase complex, leads to neural differentiation by binding to PLZF to promote the translocation of PLZF from the nucleus to the cytoplasm for ubiquitination and degradation. **(C)**: Balance between self-renewal and differentiation of spermatogonial progenitor cells (SPCs) by PLZF. Sertoli cells produce GDNF and SCF to bind the receptors on the surface of SPC, consequent activation of the phosphoinositide 3-kinase (PI3K) pathway triggers Akt, which inactivates TSC2. PLZF activates Redd1 through direct binding to distal promoter of Redd1 to induce mTORC1 inhibition in SPCs. On the contrary, depletion of PLZF results in aberrant mTORC1 activation and lowers expression of GDNF receptors (GFRα1 and c-Ret). As a result, aberrantly activated mTORC1 by depletion of PLZF inhibits the response of spermatogonia to GDNF, which leads to the loss of self-renewal of spermatogonia. In addition, PLZF also represses the expression of c-kit (receptor of SCF), which is coupled to PI3K/Akt signaling pathway to maintain spermatogenesis. **(D)**: The regulation of PLZF in osteoblast and chondrocyte differentiation of mesenchymal stem cells (MSCs). PLZF enhances the expression of Runx2 (osteogenic master regulator) during osteogenesis or Sox9 (chondrogenic master regulator) during chondrogenesis of MSCs as an upstream factor. During osteogenesis, PLZF upregulates Runx2 as an upstream factor, which directly upregulated downsteam osteogenic-specific enhancer elements including OC, OPN, Bsp, and Col1A1. During chondrogenesis, PLZF upregulates Sox9 as an upstream factor, which directly bound to and activated downsteam chondrocyte-specific enhancer elements including Col2A1, AGC1, HAPLN1, and Col11A2 in the presence of L-Sox5 and Sox6. During limb bud development, PLZF represses Hoxd, and regulates BMPs to regulate the limb patterning. Abbreviations: GDNF, glial cell derived neurotrophic factor; GFR, GDNF family receptor; HSC, hematopoietic stem cell; MSC, mesenchymal stem cell; NPC, neural progenitor cell; SCF, stem cell factor; SPC, spermatogonial progenitor cell.

large numbers of differentiated progeny. Homozygous mutations in PLZF limit the numbers of normal spermatozoa in young mice [19]. Mice lacking PLZF undergo a progressive loss of spermatogonia with age, associated with an increase in apoptosis and subsequent loss of tubule structure, but without overt differentiation defects or loss of the supporting Sertoli cells [18].

Further studies have elucidated the underlying molecular mechanisms. First of all, PLZF depletion alters the expression of genes related to metabolism, RNA binding, cell cycle, cytoskeleton as well as transcription. Deregulated expression of these genes disrupts the tight balance between spermatogonial self-renewal and differentiation. Meiotic checkpoints are

Table 1. Direct targets and cofactors of PLZF

Direct targets of PLZF				
Symbol	**Full name**	**Interaction**	**Function**	**References**
ACTA2	Smooth muscle α-actin	*	Reduces mRNA and protein levels of ACTA2, leading to a reorganization of the actin cytoskeleton	[67]
BID	BH3 interacting domain death agonist	*	Induces resistance to apoptosis in lymphocytes	[63]
CCNA2	Cyclin A2	*	Downregulates cyclin A2 to inhibit cell growth	[59, 60]
cmyc	V-myc myelocytomatosis viral oncogene homolog (avian)	*	Represses cmyc expression involved in proliferation, apoptosis and differentiation of cells	[58]
DUSP6	Dual specificity phosphatase 6	*	Induces DUSP6 to regulate myelopoiesis	[21]
ID2	Inhibitor of DNA binding 2	*	Induces ID2 to regulate myelopoiesis	[21]
IFIT2	Interferon-induced protein with tetratricopeptide repeats 2	*	Induces expression of IFIT2 for innate antiviral immunity	[53]
GFI1	Growth factor independent 1 transcription repressor	*	Inhibits GFI-1 to repress myelopoiesis	[21]
Hoxb2	Forkhead Box B2	*	Regulates development of central nervous system	[68]
Hoxd11	Homeobox D11	*	Represses Hox gene expression involved in limb morphogenesis	[65]
KIT	V-Kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	*	Maintains the pool of spermatogonial stem cells	[32]
LEF1	Lymphoid enhancer-binding factor 1	*	Inhibits LEF-1 to repress myelopoiesis	[21]
PI3K p85 α	Phosphatidylinositol-3 kinase p85 alpha subunit	*	PLZF-AT(2) complex binds to a consensus sequence of p85 alpha of PI3K to enhance its expression during cardiac hypertrophy	[72]
Redd1	DNA-damage-inducible transcript 4	*	Activates Redd1 to maintain the stemness of spermatogonial progenitor cells	[33]
RER	Renin/prorein receptor	*	Involved in proliferation and apoptosis of cardiomyoblasts	[73]
Rsad2	Radical S-Adenosyl methionine domain containing 2		Induces expression of RSAD2 for innate antiviral immunity	[53]
TpoR	Thrombopoietin receptor	*	Induces TpoR to stimulate megakaryocytic development	[34]
VLA4	Very late antigen 4	*	Controls normal and leukemic cell mobilization	[70]
Cofactors of PLZF				
AT(2)	Angiotensin II type 2 receptor	**	Interacts with AT(2) through C-terminus during cardiac hypertrophy	[72]
Bmi-1	Polycomb protein	**	Represses Hox gene expression, the important regulators of limb morphogenesis	[65]
cdc2	Cyclin-dependent kinase 1	**	Affects its stability and binding activity by phosphorylating PLZF to control cell cycle	[80]
CUL3	Cullin 3	**	Alters the ubiquitination pattern of their associated chromatin-modifying complex in the lymphoid lineage	[92]
EPN1	Epsin 1	**	Nucleocytoplasmic shuttling of epsin	[88]
GATA1	GATA binding protein 1	**	Regulates megakaryocytic development	[89]
GATA2	GATA binding protein 2	**	Stimulates the terminal differentiation of leukemic t(11;17)-associated APL blasts	[90]
Goα	Go alpha	**	Represses transcription and cell growth	[93]
HDAC1	Histone deacetylase 1	**	Forms a co-repressor complex with HDAC1 to involve in PLZF-mediated repression	[82]
HDAC4	Histone deacetylase 4	**	Involved in PLZF and PLZF-RARalpha-mediated repression	[86]
Hox5	homeobox 5	**	Restricts Shh expression in the forelimb bud	[37]
mSin3A	Mammalian Sin3 transcription regulator family member A	**	Forms a co-repressor complex with mSin3 to repress transcription	[82]
MTDH	Metadherin, LYRIC, AEG-1	**	Decreases PLZF-mediated repression, leading to evading apoptosis and increasing cell growth during tumeriogenesis	[95]
N-CoR	Nuclear receptor corepressor 1	**	Forms a co-repressor complex with N-CoR to repress transcription	[83]
RAR	Retinoic acid receptors	**	Decreases transcriptional activity of the RXR-RAR heterodimer controlling proliferation and differentiation	[91]
Rb	Retinoblastoma	**	Enhances transcription repression important for stem cells	[87]
RER	Renin/prorein receptor	**	Directly interacts with RER through protein interaction to regulate transcription	[73]
SMRT	Silencing mediator of retinoic and thyroid hormone receptors	**	Forms a co-repressor complex with SMRT to repress transcription	[83, 84]
TIMP1	TIMP Metallopeptidase Inhibitor 1	**	Significantly reduces apoptosis induced by PLZF in cervical carcinoma cells	[94]
VDR	Vitamin D(3) receptor	**	Regulates monocytic differentiation	[71]

Abbreviation: *, protein–promoter binding; **, protein–protein interaction; PLZF, promyelocytic leukemia zinc finger.

activated and apoptosis increases, leading to a progressive reduction of self-renewal capability in the spermatogonial stem cells, and thus age-dependent germ cell loss [18]. Second, PLZF directly represses the transcription of c-Kit, a hallmark of spermatogonial differentiation (Fig. 1C). The c-Kit receptor tyrosine kinase plays an important role in the postnatal stages of spermatogenesis. A point mutation in the c-Kit gene blocks the initial stages of spermatogenesis and abolishes DNA synthesis in differentiating A1-A4 spermatogonia, causing sterility [31]. A 3-bp mutation in the PLZF binding site abolishes the responsiveness of the c-Kit promoter to PLZF repression. It is consistent that PLZF knockout mice show a significant increase of c-Kit expression in their undifferentiated spermatogonia [32], suggesting that PLZF maintains the pool of spermatogonial stem cells through direct transcriptional repression of c-Kit. Thirdly, PLZF regulates Redd1 in spermatogonial progenitor cells through direct binding to its distal promoter (Fig. 1C). Redd1 opposes mammalian target of rapamycin complex 1 (mTORC1), a key mediator of cell growth. Depletion of PLZF thus activates mTORC1 aberrantly and lowers the expression of GDNF receptors, which inhibits the response of spermatogonia to GDNF, leading to the loss of self-renewal of spermatogonia [33]. These data show that PLZF is a critical rheostat for self-renewal of the spermatogonial pool.

Stem Cell Differentiation

Myeloid Differentiation

PLZF expression becomes complex in the later stages of hematopoiesis. PLZF is initially expressed in LT-HSCs but decreases during differentiation. It then reappears in more mature erythroid, monocyte, and granulocyte progenitors, but is downregulated again with terminal differentiation. During early hematopoiesis, PLZF needs to be inactivated to derepress the myeloid CD34 + lineages. Then during late myelopoiesis, PLZF is reactivated to inhibit myeloid progenitor cell growth and myeloid differentiation, leading to the accumulation of myeloid progenitor cells [23]. Thus, PLZF maintains a balance between the myeloid progenitor and mature cell compartments during normal hematopoiesis. PLZF expression is also critical for stress-induced myelopoiesis. Upon stress, ERK1/2 is activated by myeloid cytokines to suppress the activity of PLZF in myeloid progenitors through nuclear export (Fig. 1A), leading to c-Kit activation and terminal differentiation to rapidly meet the increased demand for mature, terminally differentiated myeloid cells such as erythrocytes, macrophages, neutrophils, and basophils [21].

In contrast, PLZF plays a significant stimulatory role in megakaryocytic development. PLZF progressively increases during megakaryocytic development. PLZF stimulates megakaryopoiesis, in part, by inducing the thrombopoietin receptor (TpoR) and potentiating the multiprotein transcriptional complex with GATA1 [34]. PLZF also suppresses miR-146a transcription to activate CXCR4 translation in megakaryopoiesis (Fig. 1A). In contrast, PLZF silencing impairs megakaryocytic proliferation, differentiation, and maturation [35].

Limb Bud Development

A point mutation changing the evolutionarily conserved amino acid Glu44 to Gly caused hindlimb and axial skeleton abnormalities, such as polydactyly with the formation of seven toes [36]. The absence of PLZF severely affects skeletal development in the mammalian limb. PLZF knockout mice display patterning defects, including homeotic transformations of anterior into posterior structures. In PLZF$^{-/-}$ mice, BMP expression decreases, and the Hox genes are misexpressed (Fig. 1D). Plzf acts as a growth-inhibitory and proapoptotic factor in the limb bud [17]. Just as how *C. elegans* EOR-1/PLZF cooperates with Hox genes to regulate larval cell differentiation [12], mammalian PLZF regulates Hox genes to regulate embryonic limb bud development. First of all, PLZF acts as a transcriptional repressor to directly regulate Hox gene expression (Fig. 1D). Second, PLZF interacts with Hox5 through protein–protein interaction to restrict Shh expression in the developing forelimb [37]. Shh is involved in pattern formation along the AP limb axis [38, 39]. In addition, PLZF also cooperates with Gli3 to establish the correct temporal and spatial distribution of chondrogenitors during the proximal limb patterning, which is required for all proximal cartilage condensations at very early stages of limb development. Double knockout of PLZF and Gli3 results in the apoptosis of BMPR1b-expressing mesenchymal cells at the onset of limb development [40].

Osteogenesis

PLZF is not expressed in mesenchymal stem cells (MSCs), but it is upregulated in differentiating MSCs undergoing bone formation or osteogenesis [5, 6]. As one of the most highly upregulated genes during osteogenesis of human mesenchymal stem cells (hMSCs), PLZF knockdown results in decreased expression of osteoblast-specific genes whereas PLZF overexpression improves osteogenesis of MSCs. Similar effects of PLZF overexpression on osteogenesis of immortalized MSCs are also observed [6]. Deletion of the BTB domain abrogates the effects of PLZF on osteogenesis, showing that the BTB domain plays a crucial role in osteogenesis. Runt-related transcription factor 2 (Runx2), also known as core-binding subunit-alpha 1 (CBFA1), is an essential transcription factor in the regulation of osteogenesis. Of note, PLZF modulates Runx2 expression whereas Runx2 has no effect on PLZF expression [5]. These data suggest that PLZF upregulates osteogenic master regulator Runx2, which enhances osteocyte differentiation by directly binding to downstream osteogenic genes including osteocalcin (OC), osteopontin (OPN), bone sialoprotein (Bsp), and Collagen, Type I, Alpha 1(Col1A1) [41] during osteogenesis (Fig. 1D).

Chondrogenesis

PLZF has also been shown to be functionally involved in cartilage formation or chondrogenesis. PLZF is expressed in differentiating MSCs during chondrogenesis [6, 42]. PLZF knockdown slows down chondrogenesis whereas PLZF overexpression enhances chondrogenesis. Most importantly, transplantation data showed that PLZF-overexpressing MSCs repair cartilage defects much better and faster, demonstrating PLZF's potential role in cartilage repair and regeneration [6]. Molecular studies revealed that PLZF upregulates Sox9 as an upstream factor. These data suggest PLZF upregulates the

chondrogenic master regulator Sox9, which directly binds to downstream chondrogenic genes including Collagen, Type II, Alpha 1(Col2A1) [43], aggrecan (AGC1) [44], Hyaluronan, and Proteoglycan Link Protein 1 (HAPLN1) [45] and Collagen, Type XI, Alpha 2 (Col11A2) [46] to regulate chondrogenesis (Fig. 1D).

Lymphoid Differentiation

PLZF is expressed in lymphocytes, natural killer (NK) cells, $\gamma\delta$ T cells, and a large percentage of CD8 + and CD4 + T cells [47]. In particular, PLZF plays an important role in T cell differentiation and immune development. Recent studies have shown that PLZF is essential for the development of NKT cells and other innate T lymphocytes for acquisition of their unique innate immune properties. Transgenic expression of PLZF induces the effector program to acquire effector differentiation in most CD4 + T cells [48]. PLZF expression is able to attenuate the expansion of mature, alloreactive T cells, thus suppressing graft-versus-host disease (GVHD) in T-cell allografts, suggesting PLZF-overexpressing T cells could represent a superior T-cell immunotherapy to improve cancer patient outcomes due to less GVHD and intact graft-versus-tumor effects [49]. In contrast, PLZF-deficient NKT cells fail to undergo the intrathymic expansion and effector differentiation to express NK marker and display activated phenotype due to failure to secrete large amounts of both interleukin-4 and interferon (IFN)-γ [50, 51]. Further studies revealed that PLZF expression is sufficient to promote T cell effector functions without a requirement for agonist T cell receptor (TCR) signaling [48], and independently of SAP- and Fyn-mediated signaling pathways [52].

PLZF also regulates interferon-mediated innate immunity. PLZF-deficient mice have defects in expression of specific IFN-stimulated genes and are more susceptible to viral infection. These correlate with a marked decrease in the expression of the key antiviral mediators and an impaired natural killer cell function induced by IFN [53]. In addition, PLZF limits the expression of inflammatory gene products. PLZF-deficient animals express higher levels of potent inflammatory cytokines and mount exaggerated inflammatory responses to infectious stimuli. Further analysis shows that PLZF establishes basal activity states of early response genes to maintain immune homeostasis and limit damaging inflammation [54]. In addition, knockdown of PLZF diminishes the proapoptotic phenotype of invariant natural killer T (iNKT) cells whereas overexpression of PLZF leads to the proapoptotic phenotype in T cells [55].

Cellular Function of PLZF

PLZF negatively regulates the cell cycle and apoptosis of many cell types [23, 56, 57]. Expression of PLZF results in the accumulation of cells in the G0/G1 phase and increases the incidence of apoptosis [23]. Further data show that PLZF binds the promoters of key cell cycle and proproliferative genes, such as c-Myc [58] and cyclin A2 (CCNA2) [59, 60] to repress their expression and cause growth suppression. Expression of cyclin A2 and c-Myc is able to rescue the cell cycle arrest by PLZF. In addition, PLZF can reduce phosphorylation of c-Myc by downregulating the MAPK pathway to regulate its activity [61]. PLZF also directly activates Redd1, which antagonizes the mTORC1. Suppression of mTOR results in arrest in late G1 [62] (Fig. 2A). These data suggest that PLZF controls cell growth by repressing cell cycle and proliferation genes to prevent cell cycle progression.

In contrast, a fusion protein RARa-PLZF generated in t(11;17)(q23;q21)-APL activates cyclin A2 transcription to allow expression of cyclin A and confer cells a growth advantage [59]. This change could be due to its switch in binding to the Cdc2 complex (see below). In APL, the lack of PLZF-induced cell cycle gene repression reduces the PLZF control of cell growth inhibition. In T lymphocytes, PLZF does not change the basal level of apoptosis. However, PLZF has a significant anti-apoptotic effect under serum starvation by directly binding BID, a proapoptotic member of the Bcl2 family, to decrease its expression [63]. PLZF is downregulated in human malignant mesothelioma (MM) cell line from primary tumors compared with nonmalignant mesothelial cells. Ectopic expression of PLZF in PLZF-deficient cells results in decreased cell viability, reduces colony formation, as well as increases apoptosis, indicating that downregulation of PLZF may contribute to MM pathogenesis by promoting cell survival [64].

Gene Regulatory Network of PLZF

Because PLZF is a sequence-specific transcriptional factor, its transcriptional activity or stability is delicately regulated by upstream factors, post-translational modifications and multiprotein complexes with cofactors (Fig. 2B). In addition, PLZF can form DNA loops via homodimerization to regulate target genes [65].

PLZF functions as a repressor or activator in diverse biological processes by direct binding to the specific DNA sequence of the target genes through nine Kruppel-like C_2H_2 zinc fingers. PLZF has been described as a transcriptional repressor or activator during embryogenesis [58, 59, 65]. Smooth muscle α-actin is repressed by PLZF, which causes a change of cell shape and a dramatic reorganization of the cytoskeleton [66, 67]. In addition, PLZF directly binds *Hoxb2* [68] and the highly conserved human (pro)renin receptor (PRR) [69], which has been implicated in development of central nervous system. As a transmembrane protein, PRR is the highly conserved among the species and mediates intracellular signaling involved in hypertension, diabetes and neural system. In hemopoietic cells, PLZF modulates *VLA4* expression to control normal and leukemic cell mobilization [70]. In the myeloid lineage, PLZF is coexpressed with VDR and directly binds to the DNA-binding domain (VDRE) to regulate monocytic differentiation [71]. PLZF also binds to the phosphatidylinositol-3 kinase p85 alpha subunit (p85 alpha PI3K), which is implicated in cardiac hypertrophic response [72]. PLZF regulates RER to involve in proliferation and apoptosis of cardiomyoblasts [73]. PLZF directly regulates Redd1 as a transcriptional activator to maintain the pluripotency of spermatogonia [33]. In various human solid tumors, PLZF is aberrantly overexpressed. PLZF increases tumor growth by direct binding to the *CDKN1A* promoter at the proximal Sp1-binding GC-box 5/6 and distal p53-responsive elements to repress their transcription [74]. CDKN1A, also known as p21, encodes a negative regulator of cell cycle progression. Very interestingly, PLZF also regulate proliferation and

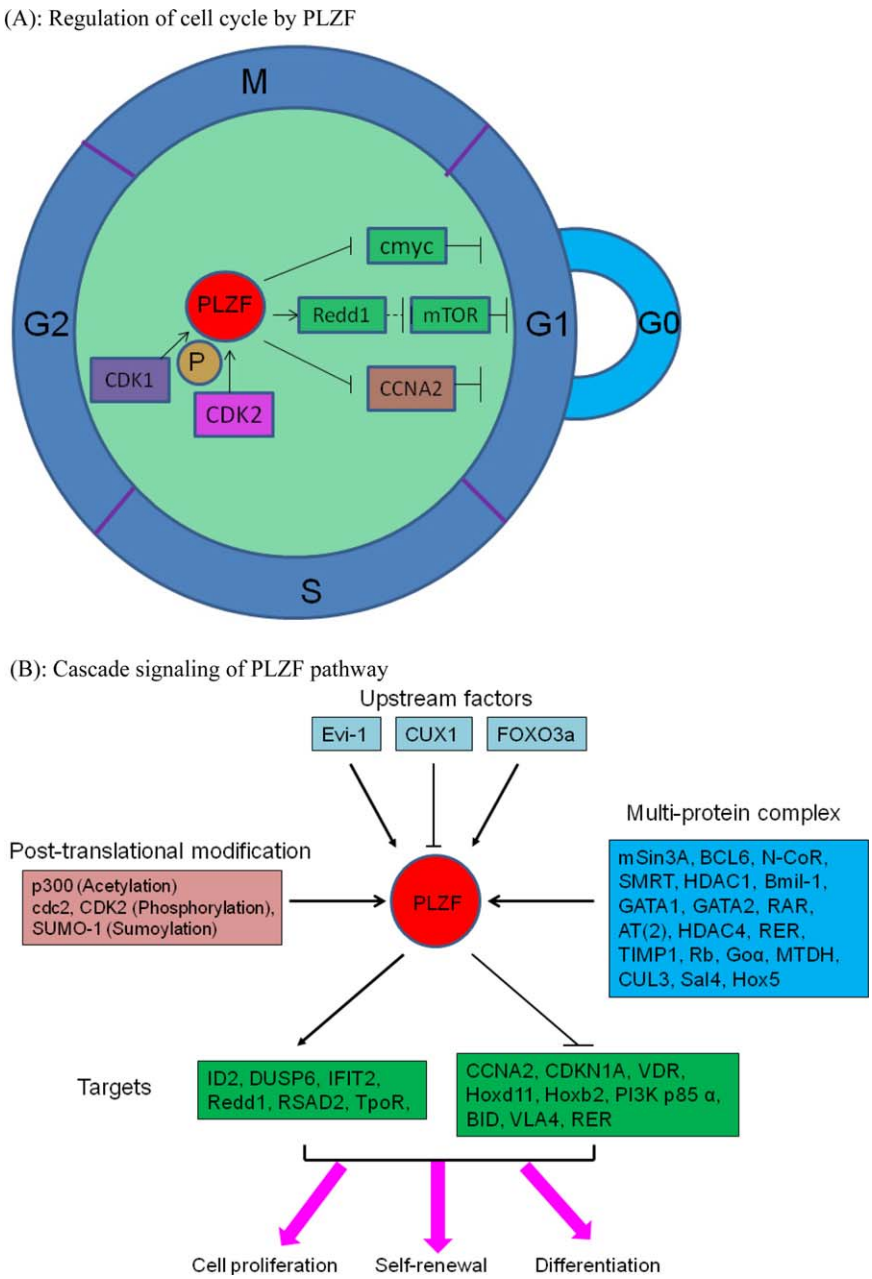

Figure 2. Molecular regulation of promyelocytic leukemia zinc finger (PLZF) in cell cycle and signaling cascade. **(A)**: Regulation of cell cycle by PLZF. PLZF inhibits cell proliferation in G0/G1 and increases apoptosis by binding the promoters of key cell cycle and proproliferative genes, such as cyclin A2 (CCNA2) and c-Myc to repress their expression, or activate Redd1 to antagonize mTOR1. Suppression of key cell cycle genes results in cell arrest in late G0/G1. At the same time, the activity or stability of PLZF is delicately regulated by phosphorylation through cell cycle kinase CDK1 (also known as CDC2) and CDK2. **(B)**: Signaling cascade of PLZF Pathway. PLZF is a transcriptional factor essential to diverse biological processes. Transcriptional activity of PLZF is delicately regulated by upstream factors, post-translational modifications, and multiprotein complexes with cofactors. Upstream factors, such as Evi-1, CUX1, and FOXO3a, regulate transcriptional activity of PLZF. Post-translational modifications, such as acetylation, phosphorylation, and sumoylation, alter the transcriptional activity or stability of PLZF. The transcriptional activity of PLZF is also regulated by multiprotein complexes with cofactors. PLZF functions as a repressor or activator by direct binding to the specific DNA sequence of the target genes.

differentiation of melanoma by directly inhibiting miR-221/222 to modulate CDKN1B and c-KIT receptor [75]. CDKN1B, also known as p27Kip1, controls the cell cycle progression at G1 and shares a limited similarity with CDK inhibitor CDKN1A.

Transcriptional activity of PLZF is delicately regulated by upstream factors. Evi-1 binds specifically to 140/-130 Evi-1-like site in the *PLZF* promoter leading to tissue-specific expression of PLZF in undifferentiated myeloid cells [76]. CUX1 binds to the 5′-UTR and promoter of *PLZF* to regulate its expression, which could be relevant to human diseases such as colorectal cancer cells and leukemia [77]. In prostate cancer, phosphorylated FOXO3a, downstream of PTEN-PI3K-AKT signaling, directly binds to the promoter of *PLZF* to inhibit prostate tumorigenesis [78] (Fig. 2B).

In addition, the activity or stability of PLZF is regulated by post-translational modifications, including acetylation, phosphorylation, and sumoylation. As a transcriptional

repressor essential to development, ability of PLZF to repress transcription is dependent on HAT activity of p300. p300 acetylates lysines in its C-terminal C2-H2 zinc finger motif to activate the PLZF binding to the promoters of target genes [79]. The activity of PLZF may also be modulated by phosphorylation through forming a high molecular complex with Cdc2. Dephosphorylation abolishes the formation of the complex, showing that phosphorylation is required for the normal function of PLZF. In contrast, the RARa-PLZF fusion does not form a complex with Cdc2 [80]. CDK2 also induces phosphorylation of PLZF, leading to ubiquitination and subsequent degradation [60]. Sumoylation by SUMO-1 at lys^{242} in the RD2 domain of human PLZF is required for transcriptional repression of PLZF, by increasing its DNA-binding affinity to its target genes, leading to growth suppression. Wild-type PLZF decreases the expression of cyclin A2 whereas sumoylation of PLZF at lys^{242} had no effects on the expression of cyclin A2 [81] (Fig. 2B). These data show that post-translational modifications are critical to the proper function of PLZF.

A third level of regulation is achieved through the formation of specific protein–protein complexes. More and more proteins are reported as cofactors to form multiprotein complexes with PLZF to regulate the activity of PLZF. It is well known that PLZF represses transcription by recruiting a histone deacetylase through the SMRT-mSin3-HDAC-NCoR corepressor complex [82–84]. HDACs participate in transcriptional repression of PLZF by deacetylating histones, resulting in local modification of chromatin structure [85]. PLZF physically interacts with HDAC1 [82], HDAC4 [86], and Rb [87] for PLZF-mediated repression. PLZF represses transcription of p53 and decreases p53 protein stability by ubiquitination through corepressor HDACs complex [74]. PLZF also interacts with Epsin 1 through amino acid 262-673 of PLZF and epsin NH(2)-terminal homology (ENTH) domain, which may be relevant to the PLZF-mediated nucleocytoplasmic shuttling of epsin [88]. Other cofactors include polycomb protein Bmi1 [65] and Hox5 [37], which are involved in limb morphogenesis; GATA1 [89], GATA2 [90], RAR [91] and CUL3 [92], which are involved in APL and differentiation of HSCs; Cdc2 [80] and Goα [93] which are involved in cell growth and cell cycle; TIMP1 [94] and MTDH [95] which are involved in apoptosis during tumorigenesis (Fig. 2B; Table 1). Altogether, these studies deepen our understanding of PLZF regulatory mechanisms in diverse biological processes and developmental contexts.

Discussion and Conclusions

Recent advances have provided a detailed picture of PLZF function as a multifunctional gene involved in diverse biological processes and diseases. Of special interest is PLZF regulating the self-renewal or differentiation of several types of stem cells, even both self-renewal and differentiation at different stages during hematopoiesis. PLZF is expressed in spermatogonial stem cells, HSCs and neural progenitors to maintain their self-renewal. In contrast, PLZF is also expressed in MSCs differentiating toward bone and cartilage, myeloid progenitors differentiating into erythrocytes, monocytes, and granulocytes, and naïve T cells differentiating into effector T cells. However, PLZF is not expressed in undifferentiated MSCs [6] and hESCs or hiPSCs (Liu TM and Lim B, unpublished data). These data suggest that the function of PLZF is context-dependent. So far, the molecular mechanisms underlying PLZF regulation of self-renewal versus differentiation in various stem cells remains poorly understood. Some questions still need be addressed, such as which signals trigger self-renewal, and which signals stimulate differentiation of stem cells. Are there common insights underlying PLZF's regulation of stemness or differentiation in different types of stem cells?

One unifying theme in PLZF's functions is that it acts as a brake on the cell cycle, to exert its effects on stem cell populations [23, 57]. Loss of PLZF often leads to the proliferative expansion of differentiated cells across metazoan evolution, such as neurogenesis from neural progenitors, spermatogenesis from spermatogonia, early myelopoiesis from LT-HSCs, and polydactyly from limb bud mesenchymal progenitors. Thus it appears to promote self-renewal in many stem cell/progenitor populations. In fact, normal PLZF represses cyclin A2 transcription, and PLZF is targeted for destruction by CDK2 complex, thus controlling the G1/S checkpoint. The PLZF-RARα, together with RARa-PLZF, causes leukemia, in part, by disrupting this function and constitutively activating cyclin A2 transcription instead. Furthermore, PLZF can suppress mTOR and c-Kit signaling to suppress cellular growth and proliferation. PLZF's reactivation during the terminal differentiation of many cell-types might represent a mechanism to repress the cell cycle and lock differentiated cells into the post-mitotic G0 state, such as during late myelopoiesis, T cell differentiation, osteogenesis, and chondrogenesis. The post-mitotic G0 state could then facilitate further differentiation to fulfill the target cell's functions. In fact, PLZF improves repair of osteochondral defects, suggesting that PLZF may represent a promising gene therapy to fully differentiate MSCs into bone and cartilage for repair and regeneration of musculoskeletal tissues. Unlike most other lineage-specific genes identified in MSCs (chondrogenesis or osteogenesis), PLZF is one of a few genes reported to improve both bone and cartilage formation, two closely related biological processes during development. The cell cycle mechanism proposed above could at least partially explain why PLZF improves osteochondral differentiation and thus repair osteochondral defects better and faster. However, the detailed molecular mechanism underlying PLZF's enhancement of osteochondral repair is still unclear, especially given that PLZF likely regulate other genes, such as Hox, Notch, MAPK/Erk, Wnt, BMP, and Hedgehog signaling, beyond cell cycle genes.

Very interestingly, PLZF can function as both a transcriptional repressor and activator. PLZF can repress transcription through the corepressor complex of SMRT-mSin3-HDAC-NCoR [82–84]. PLZF also activates the expression of Redd1 [33], TpoR [34], ID2 [21], DUSP6 [21], RSAD2 [53], and IFIT2 [53]. So far, the determinants of PLZF as an activator or repressor have not been defined molecularly. Although progress has been made in understanding PLZF function, the detailed signaling pathways and gene regulatory network underlying PLZF still remain to be elucidated. Identification of its direct transcriptional targets on a genomic scale will shed light on PLZF's molecular mechanisms. A complete understanding of PLZF, a remarkably well-conserved and broadly expressed regulator of stem cells, could very well facilitate its future applications in regenerative medicine.

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Author Contributions

T.M.L., N.S.-C., and B.L.: wrote the manuscript, generated the figures, edited and approved all figures and texts; E.H.L.: edited from early drafts to final manuscript, had significant discussions with T.M.L and participated in the final stages of writing.

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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