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The Role of PHF6 in Hematopoiesis and Hematologic Malignancies

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Abstract
Epigenetic regulation of gene expression represents an important mechanism in the maintenance of stem cell function. Alterations in epigenetic regulation contribute to the pathogenesis of hematological malignancies. Plant homeodomain finger protein 6 (PHF6) is a member of the plant homeodomain (PHD)-like zinc finger family of proteins that is involved in transcriptional regulation through the modification of the chromatin state. Germline mutation of *PHF6* is the causative genetic alteration of the X-linked mental retardation Borjeson–Forssman–Lehmann syndrome (BFLS). Somatic mutations in *PHF6* are identified in human leukemia, such as adult T-cell acute lymphoblastic leukemia (T-ALL, ~ 38%), pediatric T-ALL (~ 16%), acute myeloid leukemia (AML, ~ 3%), chronic myeloid leukemia (CML, ~ 2.5%), mixed phenotype acute leukemia (MPAL, ~ 20%), and high-grade B-cell lymphoma (HGBCL, ~ 3%). More recent studies imply an oncogenic effect of *PHF6* in B-cell acute lymphoblastic leukemia (B-ALL) and solid tumors. These data demonstrate that *PHF6* could act as a double-edged sword, either a tumor suppressor or an oncogene, in a lineage-dependent manner. However, the underlying mechanisms of PHF6 in normal hematopoiesis and leukemogenesis remain largely unknown. In this review, we summarize current knowledge of PHF6, emphasizing the role of PHF6 in hematological malignancies.

Keywords PHF6 · BFLS · T-ALL · AML · MDS/MPN · Solid tumors

PHF6 Gene and Protein

Plant homeodomain finger gene 6 (*PHF6)* is an X-linked gene (Xq26.2) transcribed into a 4.5 kb mRNA consisting of 11 exons with exons 1 and 11 representing 5′ and 3′ UTRs, respectively. Alternative splicing of *PHF6* mRNA yields two isoforms (*PHF6a* and *PHF6b*) differing in the incorporation of intron 10 to exon 11 in the longer *PHF6b* isoform [1]. A third isoform was observed in healthy individuals that showed exon 3 skipping (*PHF6*Δexon3) [2]. PHF6 is a 365 amino acid protein, highly conserved among vertebrates, and has a molecular weight of approximately 41 kDa [1, 3]. The PHF6 protein is characterized by its two atypical extended PHD (ePHD) zinc finger domains that are degenerate versions of the PZP motif (Fig. 1) [4]. The PZP motif consists of a canonical PHD finger (C4HC3), a zinc knuckle (C2HC), and an atypical PHD zinc finger (C4HC2H) [4, 5]. Each of the two nearly identical zinc fingers of PHF6 consists only of the zinc knuckle and the atypical PHD zinc finger, thereby referred to as ZaP domain, and is located at amino acid positions 14–134 (ePHD1) and 209–332 (ePHD2) in the PHF6 protein [4]. Moreover, PHF6 contains two nuclear localization sequences (NLSs), which are located at amino acids 13–16 (NLS1) and amino acids 129–133 (NLS2), and a nucleolar localization sequence (NoLS) at amino acids 157–169 of the protein (composed of NLS3 and NLS4 together) [1]. The localization of PHF6 in the nucleus and nucleolus was confirmed by subcellular localization and immunohistochemistry assay. Collectively, the PHF6 protein structure and its localization suggest a transcriptional regulatory role of the protein [1].

The sequence similarity of the PHF6 protein among humans, mice, and rats is 97.5%, and between humans and cattle, it is 99.7%, indicating a high homology in mammals [3]. In mice, the gene expression level is particularly high

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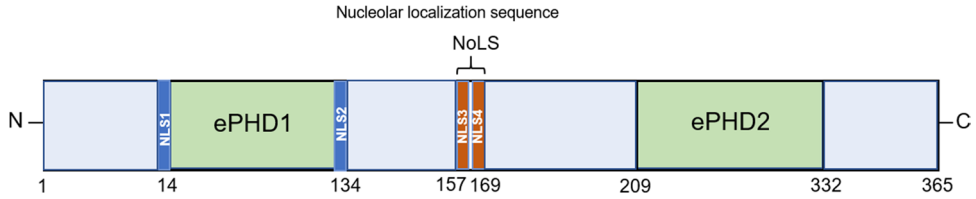


Fig. 1 PHF6 protein structure. PHF6 protein is composed of 365 amino acids with two extended PHD domains (ePHD), two nucleus localization sequences (NLSs), and a nucleolar localization sequence (NoLS) composed of NLS3 and NLS4

in the brain, anterior pituitary gland, pharyngeal arches, nasal processes, and limb buds during early development. It reduces dramatically in these tissues as development proceeds [3]. RT-PCR analysis of *Phf6* expression levels in murine myeloid progenitors and lymphoid cells showed that it is more expressed in lineage-Sca-1+cKit+ (LSK) progenitor cells as compared with common myeloid progenitors (CMP) and granulocyte–macrophage progenitors (GMPs). *Phf6* expression was highest in CD4/CD8 double-positive T-cells compared with other T-cell developmental stages and in pre-B cells compared with pro-B and mature B cells [6, 7]. Quantitative real-time PCR in human tissues showed a ubiquitous expression level of the *PHF6* gene, with the highest expression level in the thyroid, thymus, and ovaries, but moderate expression pattern in the adipose tissue, spleen, and testes [8]. Furthermore, *PHF6* is expressed in all hematopoietic subpopulations with a higher expression level in $CD34^+$ hematopoietic stem/precursor cells (HSC/HPCs). The expression level of PHF6 is moderate in $CD19^+$ B cells and $CD3^+$ T cells. In contrast, the expression in $CD56^+$ NK cells and $CD14^+$ monocytes is very low [9].

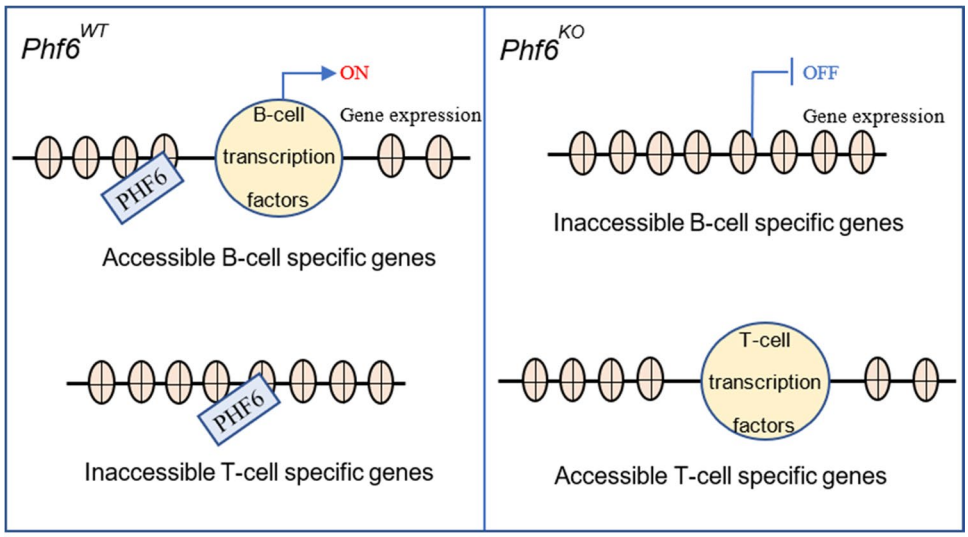
PHF6 Protein Function

PHF6 was initially thought to act as a transcription factor [10]. Studies by Feliciano et al. showed that PHF6 also functions as a chromatin state regulator and maintains a chromatin structure [11]. PHF6 interacts with core subunits of the NuRD complex proteins, including CHD4, RBBP4, and HDAC1 [4, 12]. The NuRD complex is characterized by its ATP-dependent chromatin remodeling and histone deacetylation activity, which is involved in the development and function of the brain tissue [13, 14], as well as the maintenance of HSC self-renewal and hematopoiesis regulation [15]. In vitro assays on the PHF6 extended PHD domain (PHF6-ePHD2) showed that it could bind double-stranded DNA, rather than directly binding to histone marks [12]. This is different from the canonical PHD fingers that are known for their ability to recognize histone modifications [16]. However, in B-ALL cells, the binding of PHF6 on the genome positively correlates with the binding regions of H3K27ac and H3K4me3, leading to the modulation of chromatin accessibility and nucleosome positioning in the key genes of B-cell development and maintaining B-cell identity (Fig. 2) [11]. Studies by Oh et al. demonstrated the role of PHF6 in transcriptional activation during trophectoderm lineage reprogramming, controlling placenta development. The authors found that PHF6 acts as an epigenetic reader of H2BK12 acetylation (H2BK12Ac) via its ePHD2 domain [17]. Additionally, PHF6 has an epigenetic writer role by adding monoubiquitin to the histone marker H2BK120 (H2BK120ub), thereby acting as an E3 ubiquitin ligase with UBCH3 as its E2 partner. Notably, the E3 ubiquitin ligase function of PHF6 is dependent on the preceding H2BK12 acetylation recognition [17]. These data collectively highlight the role of PHF6 as an epigenetic regulator.

Wang et al. reported that PHF6 binds to upstream binding factor (UBF) through its ePHD1 domain, and knockdown of PHF6 in HeLa cells results in cell cycle arrest at the G2/M phase, increases UBF protein levels, enhances ribosomal RNA synthesis, and promotes DNA damage at the ribosomal DNA locus [18]. The PHF6 protein binds to the transcription elongation complex and RNA polymerase II-associated factor 1 homolog (PAF1), which consists of PAF1, LEO1, CDC73, and CTR9 in the brain. This interaction, along with the downstream target gene *neuroglycan C/chondroitin sulfate proteoglycan 5 (NGC/CSPG5)*, plays a critical role in neuronal migration within the cerebral cortex. Loss of PHF6 in the brain leads to the impairment of neuronal migration within the cerebral cortex and causes the formation of heterotopia, with a consequent aberrant neuronal activity pattern possibly resulting in epilepsy [19]. In a most recent study, Warmerdam et al. found that the PHF6 protein is recruited by PARP1/2 to the sites of DNA lesions and that PHF6 is required for DNA damage repair through classical nonhomologous end joining (NHEJ) [20].

PHF6 Germline Mutations in Borjeson–Forssman–Lehmann Syndrome (BFLS)

BFLS is a rare X-linked recessive mental retardation syndrome that predominantly occurs in males, and was first reported in 1962 in three men from the same family [21, 22]. The prominent features include large ears and



Modified from Soto-Feliciano et al. *Genes and Development*. 2017

Fig. 2 Epigenetic regulation of PHF6 in B-ALL. PHF6 maintains a chromatin structure that is permissive to B-cell identity genes, but not T-cell-specific genes (left). Loss of PHF6 leads to aberrant expression of B-cell- and T-cell-specific genes resulting from lineage promiscuity and binding of T-cell transcription factors (right)

small genitalia evident from birth, hypotonia with poor feeding during infancy, truncal obesity developing in late childhood, gynecomastia (noticeable in adolescence), and coarse facial features [21]. Less common features in BFLS patients include microcephaly/macrocephaly, hypopituitarism, epilepsy, hearing impairment, Perthes disease, generalized polyneuropathy, cleft lip and palate, and short stature [23]. Germline mutation of *PHF6* is the causative genetic alteration of BFLS [1]. *PHF6* germline mutations include missense, nonsense, frameshift, duplications, and deletions [24]. Mutations distribute throughout the gene, suggesting a loss-of-function mechanism in BFLS [25].

A mouse model of BFLS was generated by substituting cysteine 99 with phenylalanine (C99F), a patient-derived mutation [29]. Phenotypic analysis of these mutant mice showed slightly reduced body weight and body length, deficits in social recognition memory, and accelerated seizure induction by the GABA antagonist pentylenetetrazol (PTZ) [29]. McRae et al. reported another BFLS mouse model with germline deletion of *Phf6* showing a significant reduction in body weight, body length, and femur length. The authors found that loss of *Phf6* downregulated the growth-hormone-releasing hormone/growth hormone/insulin-like growth factor 1 (GHRH/GH/IGF1) axis genes in the brain, pituitary gland, and liver, respectively, as compared with that in control mice [30]. Genetic deletion of the suppressor of cytokine signaling 2 (*Socs2*) using $Phf6^{-/Y}$; $Socs2^{-/-}$ double-mutant mice resulted in the elevation of growth hormone signaling and restored the growth rate in $Phf6^{-/Y}$;$Socs2^{-/-}$ mice to a comparable level as WT mice [30]. Collectively, these studies demonstrated the crucial role of PHF6 in regulating the GHRH/GH/IGF1 axis [30].

PHF6 Somatic Mutations in Hematologic Leukemia

T-Cell Acute Lymphoblastic Leukemia (T-ALL), B-Cell Lymphoma, and B-Cell Acute Lymphoblastic Leukemia (B-ALL)

T-ALL is an aggressive hematological malignancy of the thymocytes that accounts for 25% of adult and 10%–15% of pediatric ALL cases [31]. Studies by Wendorff et al. demonstrated that somatic mutation of *PHF6* is an early mutational event in the natural history of T-ALL leukemogenesis [32]. Van Vlierberghe et al. reported that *PHF6* somatic mutations were found in 38% of adults and ~ 16% of pediatric primary human T-ALLs (Table 1) [8]. In this series of *PHF6*-mutated T-ALLs, up to 70% of the mutations were nonsense and/or frameshift mutations in *PHF6* and were distributed throughout the gene [8]. In contrast, the missense mutations, which were found in 30% of the *PHF6*-mutated T-ALLs, were mainly clustered in codon C215 or the second zinc finger domain of the PHF6 protein, these findings are suggestive of a loss of function mechanism and a tumor suppressor role of the protein in T-ALL [8]. *PHF6* gene locates on the X-chromosome and its mutation was thought to be a loss-of-function mutation. In fact *Phf6* deletion in the hematopoietic system ($Phf6^{\Delta/\Delta}$) increases hematopoietic stem cell (HSC) self-renewal, $Phf6^{\Delta/\Delta}$ mice do not develop spontaneous hematologic malignancies [33, 34]. Interestingly, a recent study showed that truncated PHF6 protein can be detected in lymphoblastoid cells with a *PHF6* truncation mutation [33]. While *PHF6* mutations were originally reported predominantly in male T-ALL patients [8, 35, 36], subsequent studies from

Table 1 Ratio of PHF6 mutations in hematological malignancies

Type of Malignancy	Ratio of *PHF6* Mutations	Reference
Adult T-ALL	38%	Van Vlierberghe. *Nature Genetics* 2010 [8]
Pediatric T-ALL	16%	Van Vlierberghe. *Nature Genetics* 2010 [8]
HGBL	3%	Stengel. *Blood* 2017 [35]
T/myeloid MPAL	16%	Alexander. *Nature* 2018 [40]
MPAL	23%	Xiao. *Blood Advances* 2018 [41]
B/T MPAL	56%	Mi. *Am J Hematol* 2018 [42]
Adult AML	3%	Van Vlierberghe. *Leukemia* 2011 [35]
AML/MRC	15.4%	Mori. *Leukemia* 2016 [43]
AML/MRC	11%	Xiao. *Blood* 2017 [44]
t-AML	7.1%	Xiao. *Blood* 2017 [44]
De novo AML	3.2%	Mori. *Leukemia* 2016 [43]
Pediatric AML	2%	De Rooij. *British Journal of Haematology* 2016 [45]
CML-BC	2.47%	Li. *Leukemia & Lymphoma* 2013 [46]
CML	1.6%	Mori. *Leukemia* 2016 [43]
MDS	3%	Mori. *Leukemia* 2016 [43]
MDS/RAEB	5.3%	Mori. *Leukemia* 2016 [43]
MDS/RA	5.4%	Mori. *Leukemia* 2016 [43]
MDS/RCMD	1.3%	Mori. *Leukemia* 2016 [43]
MDS/RARS	0.9%	Mori. *Leukemia* 2016 [43]
CMML	4.7%	Mori. *Leukemia* 2016 [43]

multiple independent groups have failed to show a gender preference [6, 7, 37–39]. Despite the clinical significance, whether the truncated PHF6 exerts a loss- and/or gain-of-functions in hematologic malignancy initiation/progression remains to be explored.

Although the *PHF6* gene does not escape X-inactivation in female T-ALL patients [8], its somatic mutations are found almost exclusively in male primary T-ALL samples accompanied by aberrantly expressed *TLX1* and *TLX3* genes. However, there was no significant association between *PHF6* mutations and *NOTCH1, FBXW7,* or *PTEN* mutations [8]. In contrast, Wang et al. reported no sex difference in the prevalence of *PHF6* mutations in Chinese T-ALL patients [39]. They also showed that *PHF6* mutations are frequently co-occurring with *NOTCH1* mutations*, SET-NUP214* rearrangements, and *JAK1* mutations [39]. The complete remission rates and 1-year overall survival were comparable in T-ALL patients with or without *PHF6* mutations [39–47]. However, *PHF6*-mutated T-ALL patients do have lower lactate dehydrogenase levels and higher platelet and bone marrow blast counts than *PHF6* wild-type T-ALL patients [39].

Recurrent *PHF6* mutations were detected in 3% of high-grade B-cell lymphoma (HGBL) [48]. Although Van Vlierberghe et al analyzed 62 DNA samples from B-ALL and found no mutation in *PHF6* in these samples*,* a recent study showed that *PHF6* mutations were enriched in a pediatric *TCF3–PBX1* subgroup of B-cell precursor acute lymphoblastic leukemia and often coexist with *PAX5* mutations [8, 49]. Feliciano et al showed that *Phf6* loss in B-ALL cells results in lineage switching to T-cell specific identity genes which is speculated to result from the accessible chromatin structure upon *Phf6* loss from which the T-cell malignancies benefit [11].

Mixed-Phenotype Acute Leukemia (MPAL)

MPAL is a rare type of leukemia that has features of both myeloid and lymphoid leukemias [50]. Xiao et al. reported that *PHF6* mutation is one of the most recurrent mutations observed in MPAL [41]. Inactivating *PHF6* mutations were observed in 16% of T/myeloid MPAL patients [40]. Moreover, Eckstein et al. reported a missense mutation of *PHF6* in one T/myeloid MPAL patient with a co-occurring missense mutation in the tumor suppressor gene *PTCH1*. They also observed a nonsense *PHF6* mutation in one B/T MPAL patient with concomitant *NOTCH1*, *EZH2*, and *DNMT3A* genetic alterations [51]. Conversely, mutational analysis of MPAL from another study showed that genetic mutations of *PHF6* and *DNMT3A* were mutually exclusive [41]. *PHF6* mutations were detected in 6 out of 26 (23%) MPAL patients, and the mutations were presented as frameshift, nonsense, missense, and splicing site mutations [41]. Among the 6 *PHF6*-mutated MPAL cases, 5 of them showed T-lineage differentiation predilection. *PHF6* mutations co-occurred with other gene mutations, such as *IKZF1*, *NOTCH1 IL7R*, *RUNX1*, *SF3B1*,

JAK3, *PTPN11*, *JAK1*, *SUZ12*, *ETV6*, *ASXL1*, *FBXW7*, *WT1*, *KRAS*, and *NRAS* gene mutations, and the fusion genes, such as *PICALM-MLLT10*, *SEM4B-BCL11A*, and *SET-NUP214* [41]. Notably, *PHF6* mutations were present in all neoplastic blast populations with high variant allele frequency, implying that *PHF6* mutation is an early mutational event in MPAL [41]. Within a cohort of 9 B/T MPAL patients, 5 cases were presented with truncating mutations of the *PHF6* gene [42].

Acute Myeloid Leukemia (AML)

AML is caused by the abnormal differentiation and proliferation of myeloblasts [52]. PHF6 mutations were identified in 3% of adult AML cases [35, 43]. The PHF6 genetic lesions in hematological malignancies are largely frameshift and nonsense [8, 35]. The PHF6-mutated AML cases were classified as M0, M1, and M2 subtypes or presented as secondary AML according to the French–American–British (FAB) classification [35]. The incidence of PHF6 mutations in male AML patients is seven times more prevalent than in female AML cases [35].

PHF6 gene mutations can co-occur with other genetic lesions in AML, such as *ASXL1*, *NRAS*, *IDH2*, *FLT3*, and *CEBPα* mutations [35]. Moreover, *PHF6* mutations were detected in 4 out of 26 (15.4%) cases of AML with myelodysplasia-related changes (AML/MRC) and frequently co-occurred with *RUNX1* mutations [43]. (Mori et al., 2016). Consistently, Xiao et al. reported that *PHF6* alterations were found in 11 out of 88 (11%) AML/MRC cases and 6 out of 84 (7.1%) therapy-related AML (t-AML) cases with a primitive stem/progenitor immunophenotype and T-cell marker expression [44]. The frequently co-occurring gene mutations in de novo PHF6 mutated AML patients were *EZH2*, *SMC1A*, and *RUNX1* [43].

Two percent of pediatric AML patients harbored with somatic *PHF6* mutations. The pediatric *PHF6*-mutated AML cases were classified as FAB M0, M1, and M2 subtypes [45]. Unlike the higher ratio of male-to-female *PHF6* mutations in adult AML patients, no gender bias was observed in pediatric AML cases [45]. The *PHF6* mutations in the pediatric AML patients were mainly predicted to be loss-of-function mutations and were present as a frameshift, missense, and point mutation in an intron [45] Initial diagnosis samples from the pediatric *PHF6*-mutated AML cases showed that *PHF6* mutations were co-occurring with other gene mutations, such as *RAS*, *TET2*, *WT1*, *ETV6*, *IDH1*, and *BCORL1* gene mutations, and *RUNX1/RUNX1T1* and *NUP98/KDM5A* translocations. However, none of these co-occurring mutations were recurrent in the pediatric *PHF6*-mutated AML series [45].

Myelodysplastic Syndrome (MDS) and Myeloproliferative Neoplasms (MPNs)

CML is an MPN disorder that initiates at the hematopoietic stem cells (HSCs) caused by the chromosomal translocation t(9:22), which results in the formation of the constitutively active tyrosine kinase BCR-ABL1 protein [53]. In a cohort of 81 chronic myeloid leukemia blast crisis (CML-BC) patients, three *PHF6* mutations were identified in 2 male patients with myeloid blast crisis (2/81, ~2.47%) [46]. One of the patients who had 90% blast cells in the bone marrow harbored a frameshift mutation within exon 7 of the *PHF6* gene (p.C212Wfs X 222). The second patient had 32% blast cells within the bone marrow and had a frameshift (p.N137_E139del140fs X 142) and a missense mutation (p.A135V) within exon 5 of the *PHF6* gene. Interestingly, the frameshift and the missense *PHF6* mutations observed in the second patient were not detectable during the chronic phase of the patient's CML course. These data imply the involvement of *PHF6* mutation in the progression of CML from the chronic phase to the blast phase [46]. A clinical report by Mori et al. indicated that mutations of *PHF6* were detected in 1 out of 64 (1.6%) CML cases [43]. Furthermore, *PHF6* mutations were detected in 3% of MDS cases (34/1139 cases). They were significantly co-occurring with mutations in *U2AF1*, *RUNX1*, *IDH1/2,* and *ASXL1* [43]. More specifically, *PHF6* mutations were observed in MDS with refractory anemia with excess blasts (MDS, RAEB) (25/466, 5.3%), MDS with refractory anemia (MDS, RA) (3/49, 5.4%), MDS with refractory cytopenia with multilineage dysplasia (MDS, RCMD) (3/228, 1.3%), and MDS with refractory anemia with ringed sideroblast (MDS, RARS) (3/344, 0.9%) cases [43]. Mutations in *PHF6* were also reported in chronic myelomonocytic leukemia (a subgroup of MDS/MPN) in 4 out of 86 (4.7%) cases [43].

PHF6 Mutations in Solid Tumors

Somatic mutations of *PHF6* were observed in nonhematological malignancies as well. *PHF6* mutation was observed in a 53-year-old male hepatocellular carcinoma (HCC) patient. The mutation was reported as a nonsense mutation (c.673C>T) in exon 7, which resulted in the truncation of the full-length PHF6 protein (p.R225X) [37]. Moreover, a study by Yu et al. on primary HCC tissues showed that the *PHF6* gene expression level was significantly increased in HCC cells as compared with control noncancerous liver cells. On the other hand, the knockdown of *PHF6* in HCC cell lines resulted in reduced tumor proliferation, migration, and metastasis of the cancerous cells [54]. They also reported increased E-cadherin and reduced Vimentin expression levels upon *PHF6* knockdown. *PHF6* was also found overexpressed in patients with certain cancer, such as breast

and colorectal cancer patients) [55]. The PHF6 expression level was under-expressed in esophageal tumors [55]. These studies imply a potential oncogenic role of *PHF6* in certain types of solid tumors, and the *PHF6* gene plays different roles in a tissue-specific manner.

PHF6 Mutations Correlate with Prognosis and Drug Response

Somatic *PHF6* mutations are associated with shorter overall survival of patients with AML and MPAL [44, 56]. but do not affect the survival of T-ALL patients [47]. Xiang et al. reported that *PHF6* mutations induce resistance of T-ALL cell lines to prednisolone [57]. They further discovered that *PHF6* represses *CDKN1A* expression by directly binding and recruiting RBBP4, a component of the NuRD complex, to the promoter region of *CDKN1A*, suppressing the *p21*-mediated sensitivity of *PHF6*-mutated T-ALL cell lines to prednisolone [57]. Collectively, these studies indicate that the mutation of *PHF6* may serve as a prognostic predictor for T-ALL patients.

Phf6-Knockdown and *Phf6*-Deficient Mouse Models

Meacham et al. generated short hairpin-mediated *Phf6*-knockdown in *BCR-ABL1*+ B-ALL mouse model and found that the suppression of *Phf6* expression resulted in the impairment of B-ALL cells growth *in vivo*, suggesting a tumor-promoting role of *Phf6* in B-ALL. This data implies that PHF6 protein acts as a tumor suppressor in T-ALL and myeloid malignancies, while it acts as an oncogene in B-ALL, proposing a context-specific role of *PHF6* in hematological malignancies [58].

Several different lines of *Phf6* mouse models have been developed by multiple groups independently (Table 2). Wendorff et al. generated a conditional *Phf6* knockout ($Phf6^{KO}$) and crossed with the mice expressing *Vav1Cre* (specific for hematopoietic stem/progenitor cells) and found increased expansion of lineage-Sca-1^{+}cKit^{+} (LSK) hematopoietic progenitor cells along with increased frequencies of multipotent progenitor (MPP), MPP2, and MPP3 cell populations in the bone marrow [32]. Mixed bone marrow chimera serial transplantation assays showed a persistently higher serial repopulation capacity of $Phf6^{-/Y}$ $Rosa26^{+/Cre-ERT2}$ HSCs when compared with $Phf6^{WT}$ HSCs [32]. The chromatin accessibility profiling of *Phf6* knockout LT-HSCs by ATAC-seq (assay for transposase-accessible chromatin using sequencing) showed an increased accessibility in gene ontology term categories associated with HSC homeostasis, including ribosome biogenesis, translation, cell cycle, cellular response to stress, and MYC targets. Moreover, the RNA-seq (RNA sequencing) of *Phf6* wild-type and knockout LT-HSCs showed a differential gene expression pattern in *Phf6* knockout LT-HSCs enriched at JAK1 target genes in HSCs, suggesting a resemblance in transcriptional regulation by JAK-STAT signaling and *Phf6* inactivation in HSC self-renewal [32]. They further found that loss of *Phf6* in HSCs enhances hematopoietic reconstitution ability while inducing LT-HSC quiescence following genotoxic insult [32].

Consistently, Hsu et al. conditionally deleted *Phf6* using a *Vav1*Cre-driven approach and found that these mice had an increased number of LSK and MPP2 cell populations in the bone marrow [59]. Flow cytometric analysis of the bone marrow of the *Phf6$^{f/f}$; Vav1Cre* mice revealed an enhanced

Table 2 *Phf6*-deficient mouse models

Mouse Model	Major Phenotypes	Reference
$Phf6^{F/F}$;*vavCre*	• Increased HSC function and self-renewal capability • Increased WBC, lymphocytes, monocytes, B cells, and reduced T cells • Reduced the threshold of *NOTCH1*-induced leukemia transformation	Hsu, *Blood Adv*. 2019 [59]
$Phf6^{-/Y}$;*Vav-iCre*	■ Increased HSC function, enhanced HSC reconstitution ability ■ Reduced T-cell progenitors ■ Reduced threshold of *NOTCH1*-induced T-ALL transformation	Wendorff AA, *Cancer Discov*. 2019 [32]
$Phf6^{-/Y}$; $Rosa26^{+/Cre-ERT2}$	• Increased HSC function, enhanced HSC reconstitution ability • Reduced T-cell progenitors	Wendorff AA, *Cancer Discov*. 2019 [32]
$Phf6^{lox/Y}$;*Tie2-Cre*	• Reduced the number of HSCs • Enhanced serial transplantation capacity • Cooperated with *Tlx3* overexpression to cause penetrant early-onset lymphoid neoplasm	McRae, *Blood*. 2019 [60]
$Phf6^{fl/y}$;*Vav1-iCre*	• Increased HSC function • Slightly higher WBC in the PB caused by increased B cells	Miyagi, *Blood*. 2019 [61]
$Phf6^{fl/y}$;*Mx1-Cre*	• Increased repopulating capability of HSCs from neonatal mice	Miyagi, *Blood*. 2019 [61]
$Phf6^{fl/Y}$;*CreERT*	• Increased repopulating capability of HSCs in secondary transplantation recipient mice	Miyagi, *Blood*. 2019 [61]

cell division in MPP3 cell populations of the bone marrow [59]. The authors found that while *Phf6*-null mice develop myelodysplasia-like diseases, including reduced thrombocyte count in the peripheral blood, extramedullary hematopoiesis in the spleen, and megakaryocyte dysplasia, deletion of *Phf6* in the hematopoietic system alone is insufficient to induce leukemia [59]. Transcriptomic analysis of HSPCs from *Phf6* wild-type and *Phf6* knockout cells showed a differential expression of genes involved in stem cell differentiation, leukocyte differentiation, and cell cycle. The authors also showed that the Gtse1, Dna2, Zwilch, Plk1, and Hras genes that are known to promote cell division were upregulated in *Phf6* knockout HSPCs as compared with *Phf6* wild-type control cells [59]. Likewise, MYC and mTOR (mammalian target of rapamycin) functions and E2F1, which promote the proliferation of stem cells, were all upregulated in *Phf6* knockout HSPCs [59].

McRae et al. generated another line of conditional *Phf6* knockout mice, *Phf6*$^{lox/Y}$; *Tie*-creTg/+ mice. The Phf6-null mice also had an increased frequency of LSK HSPCs along with an increase in heterogeneous progenitor cells (HPC-1) as compared with WT mice [60], while HSCs were reduced in these *Phf6*-deficient BM cells as compared with WT cells. The authors also found that loss of *Phf6* cooperates with *Tlx3* overexpression to cause penetrant early-onset leukemia [60]. RNA-sequencing analysis of HSPCs revealed an upregulation of the interferon (IFN) α/β signaling gene signature in P*hf6*-deficient cells as compared with WT cells. RT-qPCR confirmed the enhanced expression of interferon-stimulated genes (ISGs), such as Oas2, Ligp1, and Irf7 in *Phf6*-deficient HPC-1 cell population compared with WT cells [60].

Miyagi et al. established *Phf6*f/Y; Vav1Cre and *Phf6*f/Y; Mx1Cre mice, and found that the deletion of Phf6 in embryos enhanced HSC proliferation in vitro and promoted reconstituted hematopoiesis in recipient mice [61]. They further identified that Phf6 deletion in neonates and adults resulted in an advantage in competitive repopulating capacity in vivo as measured by serial transplantation assays. Interestingly, these mice did not develop any hematological malignancies [61].

RNA-sequencing analysis using HSCs revealed that gene expression profiles differ relative to the developmental stage at which the *Phf6* gene is deleted. Gene set enrichment analysis showed a negative enrichment for negatively regulating HSC survival and proliferation signaling pathways (apoptosis pathway, transforming growth factor β signaling, and TNFα signaling) in *Phf6*-null HSCs [61]. Conversely, positive enrichment was observed in gene sets for MYC targets, E2F targets, and oxidative phosphorylation in *Phf6* knockout HSCs. When comparing the effect of the impact of TNFα on HSC proliferation, the authors found that *Phf6*-deficient HSCs are more resistant to TNFα-treatment-induced growth inhibition than WT control HSCs [61]. Moreover, chromatin immunoprecipitation followed by sequencing (ChIP-seq) of both PHF6 and the transcription factor NF-κB, a downstream effector of TNFα upon treatment with TNFα, showed that NF-κB and PHF6 peaks overlapped significantly in the leukemia cell line K562, suggesting a functional interaction between PHF6 and the NF-κB transcription factor [61].

Conclusions and Open Questions

PHF6 involves in transcriptional regulation through the modification of the chromatin state. PHF6 mutations are identified in different hematological malignancies. While PHF6 is thought to function as a tumor suppressor gene in T-ALL and AML, overexpression of Phf6 also can promote B-ALL cells, suggesting a double-edged sword of PHF6 activity, either a tumor suppressor or an oncogene, in a lineage-dependent manner. Despite the growing knowledge about PHF6 in the past years, the exact function of the protein is still unknown. The fact that *PHF6* genetic lesions in hematological malignancies are largely frameshift and nonsense, and the truncated PHF6 is detectable, leading to an open question that the truncated PHF6 could exerts a loss- and/or gain-of-functions in hematologic malignancy initiation/progression. Although it has been demonstrated that PHF6 interacts with NuRD complex, the functional role of this interaction remains elusive. Also, further experiments are needed to explain the reason underlying the higher frequency of *PHF6* mutations in hematological malignancies than in solid tumors in spite of the ubiquitous expression of the protein.

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Declarations

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