Histone-binding of DPF2 mediates its repressive role in myeloid differentiation

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Double plant homeodomain finger 2 (DPF2) is a highly evolutionarily conserved member of the d4 protein family that is ubiquitously expressed in human tissues and was recently shown to inhibit the myeloid differentiation of hematopoietic stem/progenitor and acute myelogenous leukemia cells. Here, we present the crystal structure of the tandem plant homeodomain finger domain of human DPF2 at 1.6-Å resolution. We show that DPF2 interacts with the acetylated tails of both histones 3 and 4 via bipartite binding pockets on the DPF2 surface. Blocking these interactions through targeted mutagenesis of DPF2 abolishes its recruitment to target chromatin regions as well as its ability to prevent myeloid differentiation in vivo. Our findings suggest that the histone-binding of DPF2 plays an important regulatory role in the transcriptional program that drives myeloid differentiation. X-ray crystallography | myeloid differentiation | tandem PHD finger | isothermal titration calorimetry | protein–protein interaction

The founding member of the d4 family of proteins, d4, zinc, and double plant homeodomain (PHD) finger 2 (DPF2, also known as requiem/REQ or ubi-d4), was initially discovered as a factor required for apoptosis in myeloid cells (1). d4 proteins, which in humans also include DPF1 and DPF3b, are characterized by an N-terminal requiem domain, a central C2H2-type zinc finger domain, and a C-terminal tandem PHD finger domain (2). PHD fingers, which contain two zinc finger motifs, are notable for their ability to read a diverse number of posttranslational modifications, including unmodified, methylated, or acetylated lysines, as well as unmodified arginines (3). Besides such putative binding capabilities of the DPF2 tandem PHD finger domain, relatively little is known about the regulation and function of DPF2 or its remaining individual domains. Previous studies have shown that DPF2 bridges SWI/SNF components and RelB/p52 to affect noncanonical NF-κB signaling (4), acts as a globin switching factor (5), and is a target for Staufen1–mediated mRNA decay (5). Notably, DPF2 is expressed ubiquitously in human tissues compared with DPF1 and DPF3b (6, 7). DPF2, along with DPF1 and DPF3b, has been implicated in a range of human cancers, including cervical cancer and acute myelogenous leukemia (AML) (8–13). Runt-related transcription factor 1 (RUNX1, also known as AML1) functions as an AML tumor-suppressor gene, which is frequently inactivated through somatic mutations and chromosomal translocations, including t(8;21), which produces the AML1–ETO fusion protein (14). Recent work has shown that recruitment of DPF2 into a RUNX1-containing repressor complex inhibits the expression of RUNX1 target genes, including the myeloid-specific microRNA miR-223, and inhibits myeloid differentiation (8). DPF2 recruitment appears to depend on arginine methylation events of RUNX1, as it is blocked by mutation of RUNX1 Arg223 or chemical inhibition of the type I arginine methyltransferase PRMT4. Knockdown of either DPF2 or PRMT4 increases miR-223 gene expression and myeloid differentiation. These findings suggest a model in which DPF2 and RUNX1 form a methylation-dependent repressive complex in AML, although it remains unclear whether the two proteins bind each other directly or act concertedly as part of a larger complex.

Here, we present the crystal structure of the human DPF2 tandem PHD finger domain at a 1.6-Å resolution. We demonstrate that the DPF2 tandem PHD finger domain binds acetylated H3 and H4 histone tails, identify the primary determinants of histone recognition, and confirm these interactions in vivo. We further show that a histone-binding–deficient DPF2 mutant fails to inhibit myeloid differentiation of human hematopoietic stem/progenitor cells (HSPCs), demonstrating the importance of this interaction for DPF2 function. Finally, we map the protein–protein interaction network of DPF2 in leukemia cells and find that DPF2 and RUNX1 share many common interaction partners, including components of the SWI/SNF chromatin-remodeling complex. Together, these data support the conclusion that the histone binding of DPF2 plays an important regulatory role in myeloid differentiation.

Results

Crystallization and Structure Determination of Human DPF2. Based on secondary structure prediction and sequence conservation analyses, we designed a series of N-terminal truncation constructs of Homo sapiens DPF2 for recombinant protein expression in Escherichia coli (Table S1). We identified a fragment corresponding to the tandem PHD finger domain, encompassing

Significance

Double plant homeodomain finger 2 (DPF2) is a regulator of myeloid differentiation and implicated in a range of human cancers, including acute myelogenous leukemia. Recruitment of DPF2 to chromatin has been shown to alter the expression of target genes and inhibit myeloid differentiation. Here, we present the crystal structure of the human DPF2 tandem plant homeodomain finger domain and comprehensive structure-guided biochemical and in vivo analyses. Combined, our data delineate the determinants of DPF2’s chromatin recruitment and establish its regulatory role in human hematopoietic stem/progenitor cell differentiation.

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Data deposition: The atomic coordinates have been deposited in the Protein Data Bank, www.pdb.org (PDB ID code 5VDC).

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residues 270–391, hereafter referred to as DPF2\(^\text{PHD}\). DPF2\(^\text{PHD}\) was prone to oxidation as observed by mass spectrometry and required immediate use in any experiments following purification. Crystals of DPF2\(^\text{PHD}\) were obtained in the tetragonal space group \(I4_{22}\) and diffracted to 1.6-Å resolution. The structure was solved by single-wavelength anomalous dispersion using anomalous X-ray diffraction data, taking advantage of the four endogenously bound Zn\(^{2+}\) ions per DPF2\(^\text{PHD}\) molecule. The final model contains residues 270–386, has excellent stereochemical properties, and was refined to \(R_{\text{free}}\) and \(R_{\text{work}}\) values of 18.7\% and 14.6\%, respectively. For further details of the data collection and refinement statistics, see Table S2.

Crystal Structure of DPF2\(^\text{PHD}\) Reveals a Characteristic Tandem PHD Finger Domain. DPF2\(^\text{PHD}\) contains two individual PHD finger motifs, the first comprised of residues 270–329 and the second of residues 330–386 (hereafter referred to as PHD1 and PHD2, respectively) (Fig. 1A). Each PHD finger contains the canonical architecture first described for the Williams syndrome transcription factor (Fig. 1 and Fig. S1) (15). This includes a two-stranded antiparallel \(\beta\)-sheet (\(\beta1–2\) in PHD1 and \(\beta3–4\) in PHD2) and two zinc atoms coordinated by a His-Cys motif followed by a Cys4 motif in a cross-brace topology (Cys273, Cys276, His303, Cys306 and Cys295, Cys298, Cys324, Cys327 for PHD1; and Cys330, Cys333, His353, Cys356 and Cys345, Cys348, Cys371, Cys374 for PHD2) (Fig. S1A). PHD1 and PHD2 adopt a similar conformation, with a RMSD of \(\sim 1.9\) Å over 45 Ca atoms (Fig. S1B). The two PHD fingers mainly differ in the presence of an additional short \(\alpha\)-helix in PHD1 (\(\alpha\)B, residues 311–319), resulting in three flanking helices in PHD1 (\(\alphaA\), \(\alphaB\), and \(\alphaC\)) compared with two in PHD2 (\(\alphaD\) and \(\alphaE\)) (Fig. 1B).

Evolutionary Conserved DPF2\(^\text{PHD}\) Front Surface Binds Histones in a Bipartite Manner. A multispecies sequence alignment shows DPF2\(^\text{PHD}\) to be highly evolutionarily conserved from \(H.\ sapiei\) to \(Nematostella vectensis\), with \(\sim 50\%\) sequence identity among all species tested (Fig. S2). Whereas the surface of DPF2\(^\text{PHD}\) maintains the high level of conservation observed for the entire domain, the front surface appears to be the most invariant region of the protein (Fig. 1D). This surface contains a large hydrophobic patch on its N-terminal half and a large negatively charged patch on its C-terminal half, corresponding to PHD1 and PHD2, respectively (Fig. 1C and E). Additionally, among human \(d4\) family members, DPF2\(^\text{PHD}\) shows an even greater level of conservation, with \(\sim 80\%\) sequence identity (Fig. S3A). Despite being closely related in sequence, a structural comparison of the DPF3b\(^\text{PHD}\) solution NMR structure in complex with various histone peptides and our DPF2\(^\text{PHD}\) crystal structure uncovered a movement in the relative position of the individual PHD domains, corresponding to a rotation by \(\sim 25^\circ\) (Fig. 2A) (16). Because of the high sequence identity between the two proteins, the absence of obvious differences in the PHD1–PHD2 interface, and the presence of a predicted hinge region between the two PHD finger domains, the observed conformational changes appear to be a result of ligand binding (17).
These results led us to explore the possibility that DPF2-PHD binds histone tails, possibly with a preference for post-translational modifications that differ from DPF3b. Indeed, isothermal titration calorimetry (ITC) measurements revealed that DPF2-PHD binds H3 or H4 peptides with dissociation constants (Kd) of ≈1 and ≈50 μM, respectively, but independent of their acetylation status in physiological buffer conditions (Fig. 3 A–C, Table 1, and Fig. S4 A and B). As previously described for DPF3b-PHD and consistent with the high conservation of d4 family tandem PHD finger domains, DPF2-PHD preferentially binds to H3 histone peptides (Fig. 3 B and C and Table 1) (16).

To investigate whether histone tail binding to DPF2 is governed by electrostatic interactions, we determined the dissociation constants in reduced salt conditions. In line with the electrostatic properties of the DPF2 peptide binding surface, we observed substantially tighter binding to all histone peptides in low-salt conditions (Table S3). In contrast to the histone tail interactions, we were unable to detect DPF2-PHD binding to monomethyl- or asymmetric dimethyl-arginine RUNX1 peptides, even under no-salt conditions (Table 1 and Fig. S4C). These data suggest that the previously described methylation-dependent interaction between DPF2 and RUNX1 occurs indirectly (8).

Together, our results establish that histone tail binding constitutes a common property shared among different members of the d4 family.

Identification of Essential Residues for DPF2 Histone Tail Binding. Despite extensive efforts, in all DPF2-PHD crystals we obtained, crystal packing was mediated by the same surface involved in histone tail binding, thus interfering with the determination of the complex structure. To identify individual DPF2 residues involved in histone binding, we therefore modeled the DPF2-PHD interactions with H3K14Ac and H4K16Ac using the NMR structures of DPF3b-PHD in complex with histone peptides as references (16). The resulting model suggests that histone peptides interact in a bipartite fashion with two distinct binding sites on the highly conserved front surface of DPF2 (Fig. 2B). The first binding site engages the acetyl-lysine residue with a hydrophobic pocket on the surface of PHD1, which is primarily composed of Phe275, Leu307, and Trp322. The second binding site is located on the negatively charged PHD2 surface and includes DPF2 residues Glu326 and Asp346, which make electrostatic interactions with Lys4 and Arg2 residues of the histone H3 tail, respectively.

Using the structural models of the DPF2-PHD/H3K14Ac and DPF2-PHD/H4K16Ac complexes as guides, we attempted to identify specific DPF2 residues critical for histone tail binding through site-directed mutagenesis followed by ITC. Alanine mutation of DPF2 residues Phe275 and Arg300 in the first binding site and Asp346 in the second binding site, hereafter referred to as DPF2MUT, dramatically reduced the interaction with histone tail peptides to levels that were not measurable by ITC in physiological buffer conditions (Fig. 3 B and C and Table 1). The observed loss of histone tail binding was not a result of improper DPF2MUT protein folding, as confirmed by circular dichroism (CD) spectroscopy (Fig. S5).

Together, these results pinpoint essential binding pockets in the DPF2-PHD surface that directly mediate its interactions with histone tails.

DPF2 Binds Acetylated Histones H3 and H4 in Vivo. We used our structure-guided mutational analysis as a guide to validate the interaction of DPF2 with H3 and H4 histones in vivo. First, we tested the cellular localization of FLAG-tagged DPF2 and DPF2MUT and confirmed that both proteins show identical localization patterns by cell fractionation or immunofluorescence microscopy and are predominantly localized to the nucleus (Fig. 3 D and E, Table S4). In line with our biochemical analysis, wild-type DPF2 but not DPF2MUT is able to bind H3 or H4 in vivo (Fig. 3F). However, this interaction was dependent on acetylation of H3 and H4, suggesting that additional determinants modulate the DPF2–histone interaction in an acetylation sensitive fashion in vivo. Together, these results demonstrate that DPF2 resides on Phc275, Arg300, and Asp346 within the tandem PHD finger domain are essential for its recruitment to histones.

Repression of Myeloid Differentiation Is Dependent on DPF2 Chromatin Recruitment. Having previously shown that DPF2 is recruited to the pre-miR-223 promoter region (8), we investigated whether this recruitment is mediated by the interaction of DPF2
with histones. To reduce the influence of endogenous DPF2, we overexpressed wild-type DPF2 and the histone-binding-deficient mutant DPF2*MUT in MOLM-13 cells, which have low endogenous DPF2 levels compared with other AML cell lines (Fig. 4A and B). Next, we performed ChIP assays using an anti-DPF2 antibody and primer pairs that amplify the RUNX1 binding site within the pre-miR-223 promoter region. Whereas wild-type DPF2 is primarily located at region 4 of the pre-miR-223 promoter region in MOLM-13 cells (Fig. 4C–E), DPF2*MUT displayed substantially reduced recruitment to this promoter region (P < 0.005), comparable to a non-DPF2 responsive promoter (Fig. 4D) (8). Consistently, there was no significant difference between DPF2WT and DPF2*MUT at the nonresponsive promoter (P > 0.1) (Fig. 4D).

Next, we examined the effect of DPF2 chromatin recruitment on the myeloid differentiation of human cord blood (CB) CD34+ cells by using lentiviral expression of either wild-type DPF2 or DPF2*MUT (Fig. 4F). After 7 d in myeloid differentiation-promoting cultures, the proportion of CD11b+ cells originating from wild-type DPF2-overexpressing CD34+ cells was markedly reduced, from 87.5 to 44.1%, compared with control cells. In contrast, DPF2*MUT overexpression did not reduce the number of CD11b+ cells (Fig. 4G and H). Thus, DPF2 negatively regulates myeloid differentiation and this activity is dependent on its histone binding ability.

To further understand how DPF2 regulates HSPC biology, we performed RNA sequencing of the CB CD34+ cells by using lentiviral expression of either wild-type DPF2 or DPF2*MUT (Fig. 4F). After 7 d in myeloid differentiation-promoting cultures, the proportion of CD11b+ cells originating from wild-type DPF2-overexpressing CD34+ cells was markedly reduced, from 87.5 to 44.1%, compared with control cells. In contrast, DPF2*MUT overexpression did not reduce the number of CD11b+ cells (Fig. 4G and H). Thus, DPF2 negatively regulates myeloid differentiation and this activity is dependent on its histone binding ability.

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domain-containing proteins, such as JARID1A or PHF23, have been identified in patients with AML (18–21). In both cases, the PHD domains of JARID1A or PHF23, which function as methyl-lysin recognition domains, are conserved in the fusion protein and shown to be critical for Hox gene activation and leukemia induction in vivo (22).

The tandem PHD domains of the MOZ and MORF histone acetyltransferases represent another example of histone reader domains that regulate hematopoiesis. The MOZ and MORF PHD domains recognize acetylated, crotonylated, and butyrylated H3K14, and acetylated H3K9 in vitro (23). Both acetyltransferase genes are involved in chromosomal translocations in leukemia that result in their fusion to CBP, EP300, or TIF2 (24–26).

Collectively, these studies indicate that loss of the normal recognition of histone modifications can have widespread effects on myeloid transcription and HSPC differentiation and self-renewal. The findings also suggest that targeting oncogenic epigenetic readers, such as PHD domains, could be a therapeutic strategy for treating myeloid malignancies. In one example of such an approach, chemical inhibitors that target the bromodomain of the BET family member, BRD4, have shown efficacy in treating mixed lineage leukemia gene-rearranged leukemia, as well as multiple myeloma and acute lymphoblastic leukemia (ALL) (27–29).

DPF2 is mutated in several human cancers including AML, lymphoma, and ALL (30), with mutation hot spots occurring within the N-terminal region as well as the PHD domains. Several of these N-terminal residues are predicted to be sites of posttranslational modifications, including multiple arginines that represent potential targets of the arginine methyltransferase enzymes PRMT1, PRMT4, and PRMT5 (31, 32). We, and others, have shown that myeloid differentiation is regulated by specific arginine methylation events, and PRMT4 modifies several proteins in the larger DPF2 interactome, including RUNX1 and the SWI/SNF subunit BAF155 (8, 33). Thus, the recognition of histone modifications by the PHD domains of DPF2 potentially integrates multiple epigenetic inputs and likely enables the targeting of DPF2 to specific chromatin sites.

Our data highlight the important role of DPF2 in AML; DPF2 is a potential new therapeutic target that can possibly trigger the myeloid differentiation or apoptosis of leukemia cells (Fig. 6). Although DPF2 is ubiquitously expressed, we show that...
It is overexpressed in AML cell lines, and in AML patient samples, where it represents a poor prognosis indicator (Fig. S8). Overexpression or mutation of DPF2 is expected to alter its histone-binding properties. However, further work is needed to elucidate the mechanism by which histone modifications modulate DPF2’s role in controlling myeloid differentiation, especially in light of studies implicating several histone acetyltransferases in hematopoietic differentiation and self-renewal. Nonetheless, the results presented here provide a solid foundation for further studies aiming to trigger the differentiation of AML cells through targeted manipulation of DPF2.

Methods

Details for X-ray diffraction data collection and structure refinement are described in SI Methods and Table S2. Further details of molecular cloning, protein expression, purification, crystallization, CD spectroscopy, ITC measurements, cell culture, cell fractionation, mass spectrometry, immunofluorescence microscopy, immunoprecipitation, generation of lentivirus, immunoblot analysis, flow cytometry, RNA sequencing, and ChIP assays are described in SI Methods and Tables S1 and S3–S9.

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