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REVIEW

New insights into transcriptional and leukemogenic mechanisms of AML1-ETO and E2A fusion proteins

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BACKGROUND: Nearly 15% of acute myeloid leukemia (AML) cases are caused by aberrant expression of AML1-ETO, a fusion protein generated by the t(8;21) chromosomal translocation. Since its discovery, AML1-ETO has served as a prototype to understand how leukemia fusion proteins deregulate transcription to promote leukemogenesis. Another leukemia fusion protein, E2A-Pbx1, generated by the t(1;19) translocation, is involved in acute lymphoblastic leukemias (ALLs). While AML1-ETO and E2A-Pbx1 are structurally unrelated fusion proteins, we have recently shown that a common axis, the ETO/E-protein interaction, is involved in the regulation of both fusion proteins, underscoring the importance of studying protein–protein interactions in elucidating the mechanisms of leukemia fusion proteins.
OBJECTIVE: In this review, we aim to summarize these new developments while also providing a historic overview of the related early studies.
METHODS: A total of 218 publications were reviewed in this article, a majority of which were published after 2004. We also downloaded 3D structures of AML1-ETO domains from Protein Data Bank and provided a systematic summary of their structures.
RESULTS: By reviewing the literature, we summarized early and recent findings on AML1-ETO, including its protein–protein interactions, transcriptional and leukemogenic mechanisms, as well as the recently reported involvement of ETO family corepressors in regulating the function of E2A-Pbx1.
CONCLUSION: While the recent development in genomic and structural studies has clearly demonstrated that the fusion proteins function by directly regulating transcription, a further understanding of the underlying mechanisms, including crosstalk with other transcription factors and cofactors, and the protein–protein interactions in the context of native proteins, may be necessary for the development of highly targeted drugs for leukemia therapy.

Keywords AML1-ETO, E2A-Pbx1, E-proteins, chromosomal translocation, transcription, leukemia

Introduction

All lineages of blood cells are differentiated from multipotent hematopoietic stem/progenitor cells (hereinafter referred to as HSCs). HSCs have the ability to undergo self-renewal and/or differentiation into mature lineages. These mature lineages include erythrocytes, platelets, and white blood cells. The latter contain myeloid cells, megakaryocytes, and lymphocytes (B and T cells) (Seita and Weissman, 2010). HSC differentiation is controlled by master transcriptional regulators. One such regulator is RUNX1 (also called AML1, see below), a sequence-specific transcription factor required for definitive hematopoiesis in mice (Okuda et al., 1996).

While RUNX1 has an important physiological function, it is also a target of chromosomal translocations, many of which generate leukemogenic fusion proteins (Rowley, 1999). A well-studied example is the t(8;21) translocation, which fuses the RUNX1 gene at chromosome 21 to the ETO gene at chromosome 8 (Peterson and Zhang, 2004). The t(8;21) translocation is observed in 10%–15% of total AMLs and accounts for nearly 40% of the M2 subtype (Downing, 1999; Peterson and Zhang, 2004; Reikvam et al., 2011). While the first evidence of recurring t(8;21) translocation in AMLs was reported in 1973 (Rowley, 1973), it took nearly 20 years to clone the involved genes as RUNX1 (initially named AML1, acute myeloid leukemia 1) and ETO (eight twenty-one, also called MTG8, myeloid translocation gene on chromosome 8) (Miyoshi et al., 1991; Erickson et al., 1992; Miyoshi et al.,

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1993). The translocation occurs between intron 5 of RUNX1 and intron 1 of ETO, allowing the expression of a 752-amino acid full-length fusion protein. While the first 1-177 amino acids are derived from RUNX1, the remaining 575 amino acids are derived from ETO (Fig. 1) (Peterson and Zhang, 2004). Because transcription of wild-type ETO gene is normally silenced in hematopoietic cells, it has been proposed that an important consequence of t(8;21) translocation is expression of a high level of ETO polypeptide (Chang et al., 1993; Zhang et al., 2004).

AML1-ETO has historically served as a prototype to understand how dysregulation of transcription by leukemia fusion proteins promotes leukemogenesis. In this review, we summarize the past and recent studies, focusing on protein–protein interactions, transcriptional mechanisms, and structural characterization of RUNX1/AML1-ETO domains. A new development in the field is that in leukemia cells AML1-ETO exists as a stable complex with E-proteins including HEB and E2A. Intriguingly, while E-proteins contain three members, only E2A is involved in chromosomal translocations. One of such translocations is t(1;19), which fuses the activation domain of E2A to the DNA binding domain of Pbx1 (Fig. 1). This results in the expression of E2A-Pbx1, which interferes with pre-B cell development and leads to pre-B ALLs commonly observed in children (Mellentin et al., 1989; Kamps et al., 1990 ; Nourse et al., 1990; Aspland et al., 2001). A related E2A fusion protein, E2A-HLF, generated by t(17;19) translocation, is also observed in pre-B ALLs (Inaba et al., 1992). While previous studies have focused on how E2A fusion proteins interact with coactivators to drive transcriptional activation, we have shown that the fusion proteins are also subjected to negative regulation by ETO family corepressors. Enhancing corepressor interactions may be a new strategy to target E2A fusion proteins.

Transcriptional mechanisms of AML1-ETO

Early studies have supported a dominant negative model in which the AML1-ETO fusion protein competitively binds to RUNX1 target genes and interferes with RUNX1-mediated activation. However, recent studies have shown that AML1-ETO-mediated transcriptional regulation is more complex than previously thought, as evidenced by its ability to bind to other transcription factors, to recruit not only corepressors but also coactivators, and to both repress and activate transcription in a gene-specific manner. More complexity is added by the seemingly paradoxical roles of NCoR (Nuclear Receptor Co-repressor)/SMRT (Silencing Mediator for Thyroid and Retinoid Receptors, an NCoR homolog) corepressors in regulating AML1-ETO's function. While repression is considered important for AML1-ETO-mediated leukemogenesis, removal of the strong NCoR/SMRT-binding domain does not compromise but rather enhances the leukemogenic activity of AML1-ETO. In this section, we review the literature and discuss these findings.

Early studies on RUNX1 and ETO

While the t(8;21) fusion gene at chromosome 21 was initially named AML1, it is more recently referred to as RUNX1

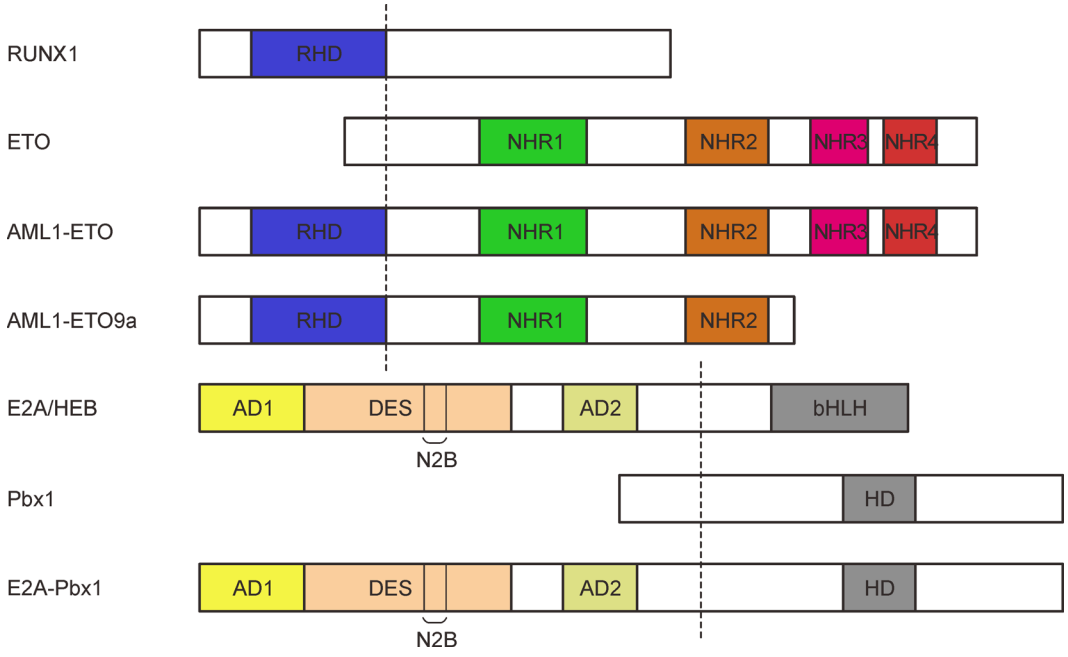

Figure 1 Schematic representations of domain organization of AML1-ETO, E2A-Pbx1, and the related wild-type proteins. RHD: runt homology domain. NHR1-4: nervy homology regions 1-4. NHR1 and NHR4 are also known as TAFH and MYND zinc fingers, respectively. AD1 and AD2: activation domains 1 and 2. DES: downstream ETO-interacting sequences, which includes a minimal binding sub-region named NHR2 binding (N2B) motif. HD: homeobox domain. bHLH: basic helix-loop-helix domain. The vertical dash lines depict the breakpoints during chromosomal translocation.

(Runt-related transcription factor 1), reflecting its similarity to *Drosophila Runt*, including the DNA binding domain RHD (Runt-homology domain) (Erickson et al., 1992). RUNX1 is also called CBFα2 as it requires dimerization with CBFβ to bind with high affinity to enhancer core motif TGTGGT (Meyers et al., 1993; Ogawa et al., 1993; Meyers et al., 1995). RUNX1 has three splicing variants: 1a, 1b and 1c, which differ in the N-terminal and C-terminal domains (Miyoshi et al., 1995). RUNX1a uniquely lacks the C-terminal transcriptional regulatory domain, allowing it to exert antagonistic effects on RUNX1-dependent gene regulation and myeloid differentiation (Tanaka et al., 1995). RUNX1 belongs to the RUNX family of transcription factors (RUNX1-3) that share the ability to bind to the RUNX motif via dimerization with CBFβ (de Bruijn and Speck, 2004; Ito, 2004). The current literature supports the notion that RUNXs have distinct biological/pathological functions, owning to their tissue/cell-specific expression patterns (Okuda et al., 1996; Zhang et al., 2000; Li et al., 2002; Lotem et al., 2015).

Cloning of ETO from t(8;21) AML immediately revealed its homology with *Drosophila* TAF110 (recently re-named as TAF4) at the N terminus and two zinc fingers at the C terminus (Erickson et al., 1994). An independent *Drosophila* screen for downstream targets of *Ultrabithorax* identified *Nervy* as the *Drosophila* homolog of ETO (Feinstein et al., 1995). Mammals possess two additional ETO-related proteins, MTGR1 (MTG8-related protein 1) (Kitabayashi et al., 1998) and ETO-2/MTG16 (myeloid translocation gene on chromosome 16) (Gamou et al., 1998). *Nervy*, ETO, MTGR1 and ETO-2 thus define a small family of evolutionarily conserved proteins. These proteins share a high degree of sequence similarity (45%-50%) at *Nervy*-Homology regions (NHR1-4) (Fig. 1). NHR1 corresponds to the aforementioned TAF4-homology domain (TAFH). NHR2 is a tetramerization domain with hydrophobic heptad repeats (HHRs) typically found in amphipathic helices (Lutterbach et al., 1998a). NHR3 displays some homology with A-kinase anchoring proteins (AKAP) (Fukuyama et al., 2001). Lastly, NHR4 is the C-terminal zinc finger domain, which is also named as MYND because of its presence in MTG8 (Myeloid translocation gene on chromosome 8), Nervy and DEAF-1 proteins (Gross and McGinnis, 1996). Underscoring the involvement of ETO family proteins in leukemias, ETO-2, like ETO, is also involved in chromosomal translocations with RUNX1, which is observed in therapy-related t(16;21)-associated AMLs (Gamou et al., 1998). While ETO expression is restricted to brain, intestine and few other tissues/cell types that exclude hematopoietic cells (Miyoshi et al., 1993; Wolford and Prochazka, 1998; Calabi et al., 2001), ETO-2 and MTGR1 appear to show broader expression patterns and are expressed in the hematopoietic compartment, including HSCs and leukemia cells (Calabi and Cilli, 1998; Fracchiolla et al., 1998; Gamou et al., 1998; Davis et al., 1999). In this regard, early reported ETO signals in $CD34^+$ cells (Erickson et al., 1996) may have resulted from antibody cross-reactivity with ETO-2 and/or MTGR1, as recently confirmed by ETO knockout studies (Calabi et al., 2001).

AML1-ETO/ETO functions in repression

While ETO cannot directly bind to DNA, it harbors potent transcriptional repression domains as initially shown by Gal4-based reporter assays (Zhang et al., 2001) and, more recently, by studies showing that ETO family corepressors serve as potent transcriptional corepressors for E-proteins (Zhang et al., 2004; Guo et al., 2009; Gow et al., 2014). In the context of AML1-ETO, early indication of its repressor function was first reported in 1995 by Hiebert and colleagues. Whereas AML1b activates transcription from the T cell receptor β enhancer, co-expression with AML1-ETO inhibits the activation in a dominant negative fashion (Meyers et al., 1995). Similar inhibition was also observed for GM-CSF (Frank et al., 1995), myeloid-specific gene defensin NP-3 (Westendorf et al., 1998) and multidrug resistance 1 (MDR-1) (Lutterbach et al., 1998a). Subsequent structure-functional studies mapped the repression activity of AML1-ETO to the NHR2-4 region of ETO (Lenny et al., 1995; Lutterbach et al., 1998a). Dominant negative inhibition of RUNX1 as a leukemogenic mechanism of AML1-ETO also gains support from animal studies showing that mice carrying a knock-in AML1-ETO allele display similar defects in hematopoiesis as the RUNX1 knockout mice (Yergeau et al., 1997; Okuda et al., 1998). However, a genetic study in *Drosophila* showed that AML1-ETO can function as a constitutive repressor independent of RUNX1 (or *lozenge,* a *Drosophila* RUNX1 homolog) (Wildonger and Mann, 2005). This result shows that the ability of AML1-ETO to regulate transcription involves not only competitive binding to DNA with RUNX1, but also a more direct mechanism to repress transcription.

A major development in the field is the discovery of direct interactions of ETO with multiple transcriptional corepressors and histone deacetylases (HDACs), which links ETO-mediated repression to regulation of chromatin structure (Gelmetti et al., 1998; Lutterbach et al., 1998b; Wang et al., 1998). In these studies, reciprocal yeast-two-hybrid screens show that ETO binds to repression domain 3 (RD3) of SMRT (Gelmetti et al., 1998) and, vice versa, NCoR binds to NHR4 of ETO (Wang et al., 1998). Sin3A corepressor, a known NCoR-interacting protein, also directly interacts with ETO via NHR2 apparently through an NCoR-independent mechanism (Fig. 2) (Lutterbach et al., 1998b). These ETO-mediated corepressor/HDAC interactions are all preserved in AML1-ETO, explaining its repressive activity. In view of the reported interactions of NCoR/SMRT/Sin3A corepressors with multiple Class-I HDACs (Hassig et al., 1997; Heinzel et al., 1997; Laherty et al., 1997; Nagy et al., 1997; Zhang et al., 1997; Guenther et al., 2000; Li et al., 2000; Zhang et al., 2002), the observed AML1-ETO/ETO-HDAC interactions may be secondary to the primary interactions with NCoR, SMRT and Sin3A (Gelmetti et al., 1998). Recently reported

genome-wide chromatin immunoprecipitation-sequencing (ChIP-Seq) studies provide functional proof for the biochemically characterized HDAC interactions by showing that depletion of AML1-ETO leads to strong and global increase of histone acetylation levels at AML1-ETO target genes (Ptasinska et al., 2012; Ptasinska et al., 2014; Trombly et al., 2015). The significance of corepressor/HDAC interactions in AML1-ETO-mediated function is also supported by studies showing that inhibiting HDAC activities by HDAC inhibitors can reverse the leukemogenic phenotypes of t(8;21) cells (Klisovic et al., 2003; Barbetti et al., 2008). However, multiple domains of ETO are involved in multivalent and cooperative interactions with corepressors and HDACs (Fig. 2) (Amann et al., 2001; Hildebrand et al., 2001). In addition, corepressors may play both positive and negative roles in regulating AML1-ETO function as revealed by the increased leukemogenic activity of the AML1-ETO9a variant (see below). It thus may prove challenging to target specifically the disease-causing corepressor interactions.

The importance of repression for AML1-ETO-mediated leukemogenesis is supported by the function of AML1-ETO-downregulated genes, such as C/EBPα and Pirin, which drive myeloid differentiation; and p14/ARF, Neurofibromatosis-1, and RASSF2, which function as tumor suppressors (Table 1). Interestingly, a previous work showed that AML1-ETO can arrest cell growth and promote apoptosis (Burel et al., 2001; Hug et al., 2002). Thus, AML1-ETO may function both as an oncogene and as a tumor suppressor. It remains to be determined whether these activities reflect different doses of corepressors or coactivators recruited to AML1-ETO or its interacting proteins, and/or its context-dependent crosstalk with AML1-ETO9a and/or RUNX1, both of which can bind to AML1-ETO target genes.

A twist in the field is that while the NHR4/MYND has been mapped to be the high-affinity binding sites for NCoR/SMRT corepressors, removal of this domain converts the full-length (FL) AML1-ETO into a much more potent leukemia fusion protein (see below). Thus, unlike the FL AML1-ETO, a truncated AML1-ETO mutant lacking NHR3/4 is capable of inducing AML in the absence of compounding mutations or second hits (Yan et al., 2004). Similarly, a naturally-occurring version of this truncation, AML1-ETO9a (Fig. 1), generated by alternative splicing that removes NHR3/4, also rapidly induces AML in mice with complete penetrance (Yan et al., 2006). Clinical significance of AML1-ETO9a is provided by its association with poor prognosis of the patients (Yan et al., 2006; Jiao et al., 2009; Li et al., 2012). Subsequent studies mapped NHR4/MYND to be responsible for the lack of an inherent leukemogenic activity in the FL protein (Ahn et al., 2008). Given that other regions of AML1-ETO also mediate corepressor interactions, one may speculate that removal of the strong NCoR/SMRT-binding site may reduce the level of

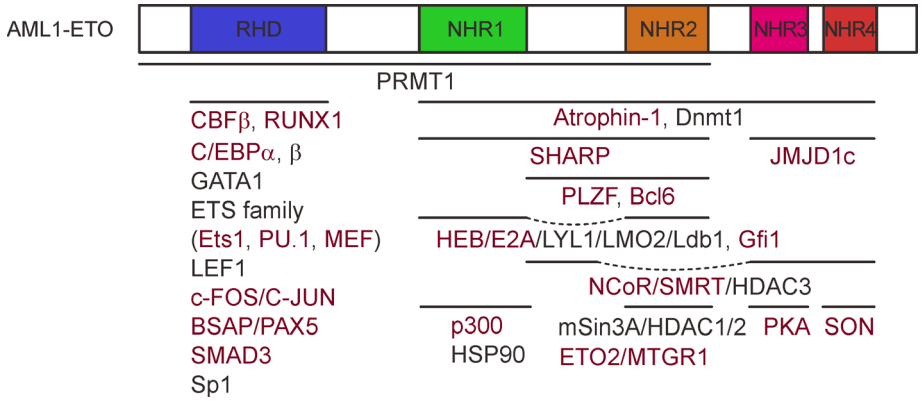

Figure 2 Domains of AML1-ETO and their interacting proteins. Biochemical studies have identified an array of AML1-ETO-interacting proteins, many of which bind to the conserved RHD and NHR domains of AML1 and ETO, respectively. Proteins bound to RHD include: CBFβ (Meyers et al., 1995), AML1(Li et al., 2015a), C/EBPα (Zhang et al., 1996), C/EBPβ (Tahirov et al., 2001), GATA1 (Elagib et al., 2003), Ets1 (Kim et al., 1999; Shrivastava et al., 2014; Shiina et al., 2015), PU.1 (Petrovick et al., 1998), MEF (Mao et al., 1999), LEF1 (Li et al., 2004), c-FOS/c-JUN(D'Alonzo et al., 2002), BSAP/PAX5 (Libermann et al., 1999), SMAD3(Jakubowiak et al., 2000), Sp1 (Wei et al., 2008). Some of the transcription factors may interact with AML1-ETO indirectly through the proximal binding sites on target DNA. Proteins bound to the ETO portion of the fusion protein include: Atrophin-1 (Wood et al., 2000), Dnmt1 (Liu et al., 2005), SHARP (Salat et al., 2008), JMJD1c (Chen et al., 2015), PLZF (Melnick et al., 2000), Bcl6 (Chevallier et al., 2004), HEB/E2A/LYL1/LMO2/Ldb1(Sun et al., 2013), Gfi1 (McGhee et al., 2003), corepressors NCoR/SMRT/HDAC3, mSin3A/HDAC1/2 (Gelmetti et al., 1998; Lutterbach et al., 1998b; Wang et al., 1998; Amann et al., 2001; Hildebrand et al., 2001; Zhang et al., 2001), p300 (Wang et al., 2011a), HSP90 (Komori et al., 1999), ETO-2/MTGR1 (Kitabayashi et al., 1998; Liu et al., 2006), PKA(RIIα) (Fukuyama et al., 2001), and SON (Ahn et al., 2008). PRMT1 binds to AML1-ETO9a fragment (Shia et al., 2012). In addition to the reported binary interactions, proteome-scale mapping of the human interactome network further showed that ETO interacts with EPS8, MEOX2, STX11, ZMYM4, LPXN, HOMER3, SPRY2, MID2, GSE1, ABI3, TAF9B, WBP11, NECAB2, PRDM14, C19orf57, CPSF7, EFHC2, LZTS2, CREB3L1, SPERT, TRIM42, CCDC36, CEP170P1 (Rual et al., 2005; Rolland et al., 2014). Proteins that have been shown to involve direct interactions are highlighted in dark red.

Table 1 List of representative AML1-ETO target genes

Repression	Molecular/Biological functions	References
C/EBPα	Transcription factor, myeloid differentiation	Pabst et al., 2001
PIRIN	Transcription factor, myeloid differentiation	Licciulli et al., 2010
P14 (ARF)	Tumor suppressor	Linggi et al., 2002
Neurofibromatosis-1	Tumor suppressor	Yang et al., 2005
RASSF2	Tumor suppressor	Samuel A Stoner, 2013
CD45	Negative regulator of JAK/STAT signaling	Lo et al., 2012
Cathepsin G	Protease, AML1-ETO degradation	Jin et al., 2013
OGG1	DNA repair	Alcalay et al., 2003
Activation	**Molecular/Biological functions**	**References**
JUP(γ-Catenin)	Wnt/β-Catenin signaling	Muller-Tidow et al., 2004; Zheng et al., 2004; Tonks et al., 2007
JAGGED1	Notch signaling	Alcalay et al., 2003
TRKA	Nerve Growth Factor receptor	Mulloy et al., 2005
ID1	Maintains HSC self-renewal	Jankovic et al., 2007
P21/WAF1	HSC self-renewal	Peterson et al., 2007
PONTIN	Cell cycle progression	Breig et al., 2014
BCL2	Apoptosis inhibitor	Klampfer et al., 1996
BCL-xl	Apoptosis inhibitor	Chou et al., 2012

NCoR/SMRT/HDAC doses bound to AML1-ETO to such an "optimal" level that eliminates the growth arrest/apoptosis activity of AML1-ETO while retaining the ability to repress other cell differentiation/tumor suppressor genes (Hess and Hug, 2004). Alternatively, other NHR4 binding proteins, such as SON (Fig. 2), a cell-cycle regulator (Ahn et al., 2011), may mediate the anti-leukemogenic function of NHR4.

Role of NHR2-mediated oligomerization

Consistent with the early mapping study showing that NHR2 can function as a potent repression domain (Lutterbach et al., 1998a), NHR2-mediated oligomerization has been shown to be important for high-affinity binding of AML1-ETO/ETO to NCoR/SMRT corepressors which is reminiscent of a similar role of nuclear receptor dimerization in recruiting NCoR/SMRT corepressors (Zamir et al., 1997; Zhang et al., 2001). Independently, NHR2 also binds to Sin3A corepressor (Hildebrand et al., 2001). NHR2-mediated oligomerization has also been shown to direct AML1-ETO to duplicated AML1 sites (Okumura et al., 2008), suggesting its impact on selective binding for specific AML1 target genes. Consistent biologic studies show that NHR2 is important for AML1-ETO's leukemogenic activities. Mutating NHR2 residues to disrupt oligomerization severely impairs the abilities of AML1-ETO to inhibit granulocyte differentiation and to stimulate clonogenicity of leukemia cells (Liu et al., 2006). Serial replating assays by Kwok et al. also showed that NHR2-mediated oligomerization is required for AML1-ETO to transform mouse HSCs *in vitro*. Intriguingly, replacing NHR2 with a heterologous oligomerization domain of FKBP restores homo-oligomerization and rescues the transforming activity of AML1-ETO (Kwok et al., 2009). Finally, animal studies showed that NHR2 is required for AML1-ETO9a-mediated leukemogenesis (Yan et al., 2009). Studies of PML-RARα support the notion that oligomerization of leukemia fusion proteins may be a common mechanism of leukemogenesis (Minucci et al., 2000). These results establish NHR2 as an important therapeutic target.

AML1-ETO functions in activation

While AML1-ETO and ETO share the ability to repress transcription, AML1-ETO also gains a unique ability to activate transcription of target genes (Table 1). Unlike certain other transcription factors such as nuclear hormone receptors, AML1-ETO specifically represses and activates distinct genes, a signature that may be useful for diagnosis and treatment of t(8;21) AML (Ross et al., 2004; Valk et al., 2004). AML1-ETO-repressed and-activated genes also show overlapping and specific functions. Whereas apoptosis genes are shared by both categories, AML1-ETO appears to specifically repress differentiation genes, while activating self-renewal genes, such as JUP/plakoglobin/γ-Catenin, which activates Wnt/β-catenin pathway (Müller-Tidow et al., 2004; Zheng et al., 2004; Tonks et al., 2007), and Jagged1, an activator of Notch signaling (Alcalay et al., 2003). These results support multifaceted involvement of AML1-ETO in diverse pathways, including inhibition of cell differentiation, (positive and negative) regulation of apoptosis, and stimulation of self-renewal of HSCs. The latter, initially reported by several groups (Hug et al., 2002; Mulloy et al., 2002; Alcalay et al., 2003), is considered a major leukemogenic activity of AML1-ETO, which increases the pool of HSCs thereby predisposing these cells to further leukemogenic hits toward productive leukemogenesis. It should be noted that AML1-ETO target genes have been recently expanded to microRNA-coding genes, which also play important roles in AML1-

ETO-mediated leukemogenesis (Fazi et al., 2007; Li et al., 2008; Chen et al., 2010; Li et al., 2013; Li et al., 2015b).

The mechanism of activation by AML1-ETO was largely elusive until several recent reports of AML1-ETO interactions with coactivators. AML1-ETO and AML1 synergistically activate the M-CSF receptor promoter (Rhoades et al., 1996). Deletion of NHR1 renders AML1-ETO much less capable of activating the M-CSFR promoter. The p300 histone acetyltransferase (HAT) directly binds to AML1-ETO via NHR1 (Fig. 2) and mediates activation of diverse AML1-ETO target genes, including p21/WAF1, Id1, and Egr1 (Wang et al., 2011a). Mechanistically, Lys24 and Lys43 in the context of AML1-ETO and AML1-ETO9a were acetylated by p300, a PTM required for AML1-ETO9a-mediated leukemogenesis in mice. While Lys43 acetylation may facilitate subsequent transcriptional activation by recruiting the TFIID general transcription factor, it is also possible that acetylation may increase the DNA binding affinity of AML1-ETO, given the reported similar effect on RUNX1 (Yamaguchi et al., 2004), along with the effect of lysine acetylation on DNA binding by p53 (Gu and Roeder, 1997; Tang et al., 2008). In another work, PRMT1, a protein/histone arginine methyltransferase, was shown to bind to and promote transcriptional activation by AML1-ETO (Fig. 2). Underscoring the biologic importance of these interactions, both p300 and PRMT1 have been shown to be important for AML1-ETO/AML1-ETO9a-mediated oncogenic activities in vitro and in vivo (Wang et al., 2011a; Shia et al., 2012). Adding to the list of AML1-ETO co-activators, JMJD1C, a histone H3K9 demethylase, was shown to bind to (Fig. 2) and mediate AML1-ETO-dependent activation of target genes (Chen et al., 2015). JMJD1C may play a broader role in leukemogenesis given its requirement for survival of multiple human AML cell lines (Chen et al., 2015).

An unsolved mystery is how AML1-ETO distinguishes its targets to mediate activation or repression. A recent work provided some insight into this important question. It was shown that RUNX1 and AML1-ETO bind to each other and occupy adjacent but distinct motifs across the genome. The relative binding signals of RUNX1 and AML1-ETO appear to determine whether the genes are activated or repressed by AML1-ETO. Activated genes show more RUNX1 binding, and the AML1-ETO/RUNX1 complex was found to recruit AP1 to mediate transcription activation (Li et al., 2015a).

Genetic events that cooperate with AML1-ETO/AML1-ETO9a in t(8;21) leukemogenesis

Early studies using mouse models showed that AML1-ETO alone cannot induce leukemias, implying that additional mutations or secondary hits are required (Rhoades et al., 2000; Yuan et al., 2001; de Guzman et al., 2002; Higuchi et al., 2002; Mulloy et al., 2003; Fenske et al., 2004). Mutations or secondary hits that affect ASXL1, ASXL2, FLT3, KIT, NPM1, MLL, IDH1, IDH2, KRAS, NRAS, CBL, CEBPA, WT1, DNMT3A, TET2 and JAK2 have been detected in t(8;21) patients, with KIT and NRAS being the most commonly affected genes (Goemans et al., 2005; Shen et al., 2011; Krauth et al., 2014; Micol et al., 2014). Mouse models have shown that many of these secondary events can indeed cooperate with AML1-ETO and AML1-ETO9a oncogene to promote leukemogenesis.

A large cluster of mutations and secondary events directly or indirectly affect components of signal transduction pathways. Examples include activating mutations in receptor tyrosine kinases such as FLT3 (Fms-like Tyrosine Kinase 3) (Schessl et al., 2005), c-KIT (Wang et al., 2011b; Li et al., 2015b) and TEL/PDGFbetaR (Grisolano et al., 2003). Additionally, a NRAS G12D mutation has been reported to facilitate a stepwise process that accelerates leukemia onset (Zuber et al., 2009; Chou et al., 2011). Two studies reported the involvement of AKT pathways. Id1 has been reported to directly bind AKT and modulates its signaling activity (Wang et al., 2015). Deletion of Id1 gene postponed leukemogenesis in AML1-ETO9a-tranduced mice. PTPN11 is a phosphatase acting downstream of tyrosine kinases and its D61Y mutation cooperates with AML1-ETO to induce leukemia (Hatlen et al., 2016). These changes lead to constitutive signaling through the RAF/MFK/FRK and PI3K/AKT pathways (Goemans et al., 2005; Scholl et al., 2008; Chou et al., 2011; Li et al., 2013; Yohe, 2015).

Another cluster of the mutations and secondary events affect regulators of DNA methylation. AML1-ETO is able to induce leukemia in a TET2 deficient background (Rasmussen et al., 2015; Hatlen et al., 2016). Mutations in IDH1/2 and loss-of-function mutations in WT1 also attenuate TET2 function and reduce DNA hydroxymethylation in vivo (Figueroa et al., 2010; Rampal et al., 2014). HIF1α, a hypoxia-induced transcription factor, has been shown to cooperate with AML1-ETO to promote leukemogenesis by inducing the expression of DNMT3a, a DNA methyltransferase enzyme (Gao et al., 2015; Yohe, 2015).

A few other mutations and secondary events affect tumor suppressor, cell cycle regulator and transcription factor proteins. AML1-ETO rapidly induced leukemia when WT1 (William tumor protein) was overexpressed (Nishida et al., 2006). Loss of p21/CDKN1A/CIP1/WAF1 also facilitated AML1-ETO-induced leukemogenesis (Peterson et al., 2007). In addition to HIF1α, a recent study showed that the hematopoietic transcription factor ZBTB7A was frequently mutated in t(8;21) patients, which abolished its DNA binding activity (Hartmann et al., 2016). E-proteins also fall into this category. While the role of E-proteins in AML1-ETO-mediated leukemogenesis will be discussed in more detail in the next section, depletion of E-proteins has been shown to delay the development of t(8;21) AML in mouse models, indicating that E-proteins function as AML1-ETO cooperative factors (Sun et al., 2013).

ETO/E-protein axis in leukemogenesis and hematopoiesis

Discovery and biochemical characterization of the ETO/E-protein axis

Biochemical purification coupled with mass spectrometric identification of proteins bound to ETO identified E-proteins as stoichiometric components of AML1-ETO-containing protein complexes (Zhang et al., 2004). E-proteins, which include HEB, E2A and E2-2, comprise a family of ubiquitously-expressed basic helix–loop–helix (bHLH) transcription factors that recognize the E-box element (CANNTG) (Massari and Murre, 2000). While all three E-protein members have the ability to bind to AML1-ETO (Zhang et al., 2004; Guo et al., 2009; Gow et al., 2014), AML1-ETO associates predominately with HEB and E2A, presumably reflecting their high-level expression in hematopoietic cells. In fact, Both HEB and E2A play important roles in regulating multiple hematopoietic pathways, including lymphopoiesis (Quong et al., 2002; Kee, 2009; de Pooter and Kee, 2010), erythropoiesis (Anantharaman et al., 2011), and development of megakaryocytes (Hamlett et al., 2008) and dendritic cells (Cisse et al., 2008). E-proteins, in particular E2A, also show cell-autonomous functions by acting as tumor suppressors and regulators of cell cycle (Engel and Murre, 1999; Zhao et al., 2001; Schwartz et al., 2006) and apoptosis (Park et al., 1999; Toyonaga et al., 2009).

At least three binding surfaces exist between AML1-ETO/ETO and E-proteins. These include the initially reported binding surface between NHR1 of ETO and AD1 (activation domain 1) of E-proteins (Zhang et al., 2004), and a subsequently identified surface between NHR2 of ETO and the DES (downstream ETO-interacting sequence) domain of E-proteins (Guo et al., 2009). A weak, yet specific interaction also exists between DES and NHR1 (Fig. 3) (Guo et al., 2009). These interactions cooperatively mediate the strong binding between E-proteins and AML1-ETO/ETO. Conserved motifs have been shown to mediate these interactions. Within AD1, PCET (p300/CBP and ETO target) mediates interactions with AML1-ETO/ETO corepressors and p300/CBP coactivators in a mutually exclusive fashion (Zhang et al., 2004). In addition to AD1, p300 also binds to AD2 of E-proteins in a manner cooperative with its binding to AD1 (Bayly et al., 2004). It has been shown that the multiple interactions of E-proteins with AML1-ETO/ETO are important for the displacement of p300 from E-proteins, thereby repressing transcription (Guo et al., 2009).

Role of ETO/E-protein axis in t(8;21) AML

Genome-wide ChIP-Seq studies demonstrated that AML1-ETO co-localizes with HEB at all their targets (Gardini et al., 2008; Martens et al., 2012; Sun et al., 2013; Ptasinska et al., 2014). These targets are enriched with both Runx and E-box elements, consistent with the notion that AML1-ETO and E-proteins selectively and cooperatively bind to DNA that contain both elements. In biological studies, while all findings are consistent with a critical importance of NHR2 (which may occur through its ability to mediate oligomerization and/or binding to DES), there are seemingly conflicting results regarding the role of NHR1. Several studies using serial replating or mouse models showed that disrupting only the PCET-NHR1 interaction via deletion or point mutations is not sufficient to abolish the oncogenic function of AML1-ETO or AML1-ETO9a (Kwok et al., 2009; Park et al., 2009a; Yan et al., 2009). However, these studies may not eliminate the "third" interaction between NHR1 and DES mentioned above, which also synergizes with the NHR2/DES interaction to achieve a strong E-protein/ETO interaction (Guo et al., 2009). Indeed, Nimer and colleagues showed that a larger deletion of NHR1 that removes this third interaction strongly reduces the leukemogenic function of AML1-ETO (Wang et al., 2011a). By using a 3D structure model (see below), Roeder and colleagues were able to differentiate NHR2 residues involved in DES binding versus oligomerization. They showed that disruption of NHR2 interaction with DES, but not NHR2-mediated oligomerization, severely compromises the ability of AML1-ETO9a to induce leukemia development (Sun et al., 2013). Depletion of E-proteins also delays leukemia development. These studies unequivocally support a critical contribution of E-proteins to AML1-ETO-mediated leukemogenesis.

The function of E-proteins extends beyond their interactions with AML1-ETO to recruiting additional factors/cofactors to AML1-ETO on target genes. Thus, AML1-ETO and E-proteins nucleate the formation of a multi-protein complex termed AETFC (AML1-ETO-containing transcription factor complex). This complex also contains CBFβ, ETO-2, MTGR1, the tissue-specific Class-II bHLH factor

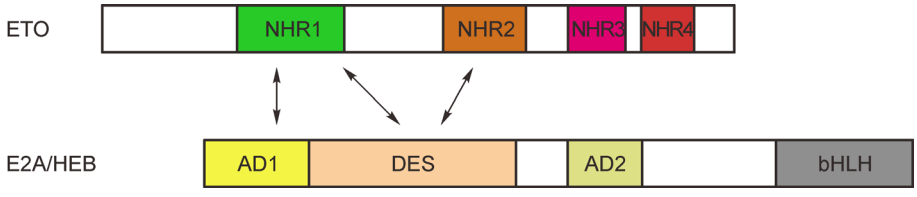

Figure 3 ETO and E-protein interactions. Schematic representations showing domain structures of ETO and E-proteins, along with the three binding interfaces: NHR1/AD1, NHR2/DES and NHR1/DES (Guo et al., 2009). DES harbors the minimal sub-region that shows binding to NHR2 (N2B).

LYL1, and the LIM-domain protein LMO2 and its binding partner Ldb1 (Fig. 2) (Sun et al., 2013). Consistent with a bridging role for E-proteins in AML1-ETO interactions with other components of the complex, the E-protein bHLH domains interact with both Class-II factors and LMO2/Ldb1 on E-box motifs (El Omari et al., 2013). The biologic importance of the multi-protein complex was underscored by findings that these subunits co-occupy and co-regulate AML1-ETO target genes and knockdown of these subunits delays AML1-ETO9a-induced leukemogenesis (Sun et al., 2013). Collectively, these studies show that the ability of t (8;21) fusion proteins to interact with E-proteins is necessary for their abilities to deregulate gene transcription and promote leukemogenesis. Although these results support the notion that interactions with E-proteins serve the purpose for fusion proteins to inhibit E-protein's pro-differentiation and tumor suppression functions (Zhang et al., 2004), they do not prove that disrupting the normal transcriptional programs directed by E-proteins is sufficient to cause AML, which is also shown by the failure of E2A-deficient mice to develop AML. These mice, instead, develop ALL (Bain et al., 1997; Yan et al., 1997) and re-introducing E2A into the ALLs cause profound apoptosis (Engel and Murre, 1999).

The current literature is consistent with the model that the t (8;21) fusion proteins hijack E-proteins to re-program the transcriptional landscape of t(8;21) cells which results in leukemogenesis. Accordingly, in t(8;21) cells, E-proteins may have lost their "normal" tumor suppressor function and, instead, become cooperative factors of the fusion proteins. In this regard, a previous work from Kamp and colleagues showed that AML1-ETO can block the differentiation that occurred upon removal of conditionally expressed E2A-Pbx1 fusion protein in a myeloid cell line (Sykes and Kamps, 2001). While this study shows converge between different leukemogenic pathways, it also lends support to the idea that AML1-ETO expression can dominantly suppress the tumor suppressor functions of E2A that may possibly arise upon the removal of E2A-Pbx1. It is reasonable to believe that t(8;21) leukemic cells are addicted to the interactions between fusion proteins and E-proteins in order to maintain their malignant phenotypes. Therefore, targeting these interactions to segregate fusion proteins from endogenous E-proteins should prove to be a promising strategy for the development of targeted therapies for t(8;21) AML. Given that wild-type E-proteins may have functioned as cooperative factors of AML1-ETO in t(8;21) cells, introducing E-proteins into these cells may not achieve the desired tumor suppression effect. Thus, successful implementation of the targeting strategy may require introducing E-protein fragments that contain only the ETO-interacting domain but lack the DNA binding domain. Such fragments from HEB may prove to be useful given its demonstrated stronger interaction with ETO compared to that of E2A. Given the recent advance in the drug design and delivery (Azzarito et al., 2013; Higueruelo et al., 2013; Arkin et al., 2014; Laraia et al., 2015), developing small chemicals and peptidomimetics inhibitors that mimic these E-protein fragments to specifically block E-protein/AML1-ETO interactions are likely to be useful drugs for the treatment of t (8;21) AML.

Role of ETO/E-protein axis in other leukemogenic and normal hematopoiesis pathways

Previous studies have established functional importance of the E2A activation domain-mediated transcriptional activation for E2A-Pbx1's leukemogenic function (Kamps and Baltimore, 1993; Lu et al., 1994; Geng et al., 2012). Both E2A and E2A-Pbx1 recruit p300/CBP via direct and cooperative binding via AD1 and AD2 (Bayly et al., 2004; Denis et al., 2014). AD1 in this context appears to be especially important given that a single mutation of a conserved Leu within PCET, which disrupts p300 interaction, also disrupts the leukemogenic function of E2A-Pbx1 (Bayly et al., 2006).

We reported a striking difference between E2A-AD1 and HEB-AD1 in the ability to bind ETO family corepressors including ETO-2. Remarkably, replacing E2A-AD1 with HEB-AD1 in the context of E2A-Pbx1 completely abolishes its abilities to mediate transcriptional activation of key target genes and to transform cells (Gow et al., 2014). This has been attributed to three amino acid differences at the C terminus of PCET between E2A-AD1 and HEB-AD1 (Fig. 4A), which specifically weaken E2A-AD1-corepressor interaction but not E2A-AD1-coactivator interaction. Thus, the transcriptional and oncogenic functions of E2A-Pbx1 not only depend on its ability to recruit p300, but also depend on its ability to bypass interactions with ETO corepressors such as ETO-2, which is expressed at high levels in B cells. The changes of E2A-AD1 sequence, however, do not completely abolish the sensitivity of E2A to repression by ETO corepressors. In t(8;21) cells, a high-level expression of AML1-ETO overcomes the reduced binding affinity with E2A, allowing AML1-ETO to bind and consequently suppress E2A target genes involved in tumor suppressor function (Engel and Murre, 1999; Gow et al., 2014). In t(1;19) leukemia cells, E2A-Pbx1 exploits the unique feature of the E2A-AD1 sequence to bypass ETO-2 interaction and repression, thereby ensuring activation of the oncogenic E2A-Pbx1 target genes to promote leukemogenesis (Fig. 4B,C) (Lu et al., 1995; Gow et al., 2014). These studies reveal context-dependent roles of ETO/E-protein interactions in distinct leukemogenic pathways, and highlight the importance of understanding the mechanisms of individual leukemia fusion proteins for effective targeting in therapeutic interventions.

ETO-2/E-protein interaction also facilitates the formation of a multi-protein corepressor complex containing ETO-2, MTGR1, E2A/HEB, TAL1/SCL, LMO2, Ldb1, and GATA1 (Schuh et al., 2005; Goardon et al., 2006). In this complex,

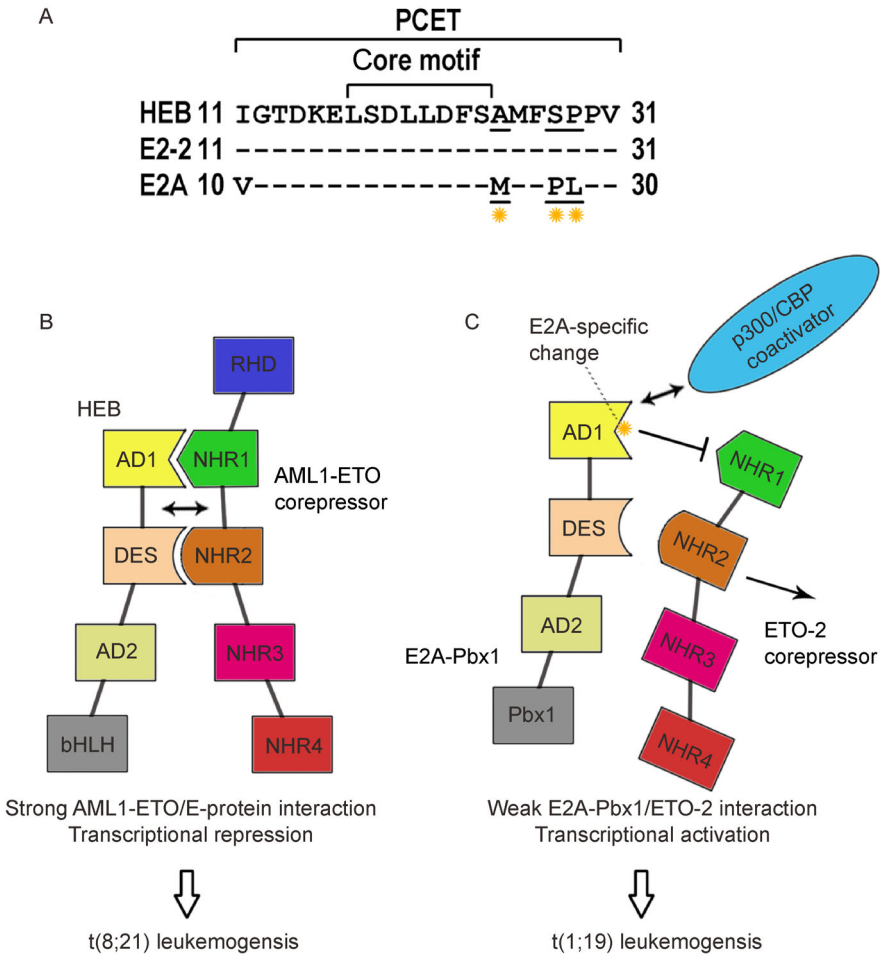

Figure 4 Differential involvement of ETO-corepressor/E-protein interactions in distinct leukemogenic pathways. (A) Alignment of PCET sequences from HEB, E2-2 and E2A. E2A-specific change is labeled with yellow stars. (B) In t(8;21) AML, strong AML1-ETO/E-protein (HEB or E2A) interactions facilitate transcriptional repression to promote leukemogenesis. (C) In t(1;19) ALL, due to E2A-specific amino-acid changes flanking the PCET motif, the binding affinity of E2A-Pbx1 to ETO-2/MTGR1 corepressors is reduced. As a result, E2A-Pbx1 is skewed toward recruiting p300/CBP HATs for gene activation.

ETO-2 serves a gatekeeper role by repressing E-protein/TAL1/GATA1 target genes, thereby preventing the premature differentiation into erythrocytes and megakaryocytes (Goardon et al., 2006; Hamlett et al., 2008). ETO-2 also plays an important role in regulating HSCs. An early study showed that ETO-2 is indispensable for definitive hematopoiesis in zebrafish (Meier et al., 2006). Subsequent studies showed that ETO-2 is required for cell fate decision and proliferation of early HSCs in mice (Chyla et al., 2008). ETO-2$^{-/-}$ bone marrow cells fail to transplant recipient mice due to a loss of self-renewal activity (Fischer et al., 2012). Consistently, ETO-2-deficient long-term HSCs showed cell-cycle blockage at the S phase (Fischer et al., 2012). A recent work showed that loss of ETO-2 compromises T cell development (Hunt et al., 2011). While this finding is consistent with ETO-2 regulation of E-protein's transcriptional activities, it seems incompatible with a corepressor function of ETO-2, given that E-proteins are positive regulators of T cell development. It is possible that the observed defects in T cell development reflect a defect of HSCs given the aforementioned role of ETO-2 in HSC regulation, along with the diminished Notch activity in ETO-2-deficient cells (Chyla et al., 2008; Hunt et al., 2011).

Structural insights into AML1-ETO functions

The structure of the RHD domain has been studied in the context of RUNX1, whereas ETO domain structures have only been reported recently. In this section, we provide a summary of these studies (Table 2), along with a brief discussion of their functional implications.

Runt-homology domain structures

Both free and complexed forms of RHD with and without DNA have been reported (Table 2). Overall, RHD assumes an

Table 2 Reported 3D structures of AML1-ETO domains

Domain	Structure description	PDB number	References
RHD	RHD	1CO1, 1LJM, 1CMO	Berardi et al., 1999; Nagata et al., 1999; Bartfeld et al., 2002
	RHD + DNA	1HJC	Tahirov et al., 2001
	RHD + CBFβ + C/EBPβ + DNA	1IO4	Tahirov et al., 2001
	RHD + CBFβ + DNA	1H9D	Bravo et al., 2001
	RHD + CBFβ	1E50	Warren et al., 2000
NHR1(eTAFH)	NHR1	2H7B	Plevin et al., 2006
	NHR1 (F136Y)	2PP4	Wei et al., 2007
	NHR1 + HEB-PCET	2KNH	Park et al., 2009a
NHR2	NHR2	1WQ6	Liu et al., 2006
	NHR2 + HEB-N2B	4JOL	Sun et al., 2013
NHR3	NHR3 + PKA(RIIα)	2KYG	Corpora et al., 2010
NHR4	NHR4	2OD1	Liu et al., 2007
	NHR4 + SMRT	2ODD	Liu et al., 2007
dTAFH	TAF4-TAFH	2P6V	Wang et al., 2007

all-β structure characteristic of the S-type immunoglobulin (Ig) fold that is also observed in DNA binding domains of p53 and NFκB (Cho et al., 1994; Chen et al., 1998; Nagata et al., 1999). RHD binds to DNA through the bottom loops and the C-terminal tail (Bravo et al., 2001; Tahirov et al., 2001). The opposite side of the structure mediates interactions with CBFβ, which consists of a β-sandwich surrounded by 4 α-helices (Fig. 5) (Huang et al., 1999; Warren et al., 2000; Bravo et al., 2001; Tahirov et al., 2001). These structural studies explain how CBFβ increases the DNA binding affinity of RUNX1 and indicate that CBFβ may play a similar role in facilitating DNA binding by AML1-ETO, which is required for its leukemogenic function (Matheny et al., 2007; Yan et al., 2009). There are, however, conflicting reports on the role of CBFβ in AML1-ETO's leukemogenic activity (Kwok et al., 2009; Park et al., 2009b; Roudaia et al., 2009; Kwok et al., 2010). These discrepancies may result from the use of different assays and their different sensitivities to the change of binding affinity between AML1-ETO and CBFβ.

NHR1/eTAFH domain structures

Three NHR1-containing structures have been solved by nuclear magnetic resonance (NMR) (Plevin et al., 2006; Wei et al., 2007; Park et al., 2009a) (Table 2). NHR1 folds into a four-helix bundle (α1-α4) structure. This fold is similar to the paired amphipathic helix (PAH) domain of Sin3A (Plevin et al., 2006). Although the overall folding of NHR1 is similar among the three reported structures, the C-termini of α4 differ significantly, suggesting that it may have a flexible conformation in native proteins. The PCET binding site is located at a grove formed by α1 and α4 helices. Three conserved Leu

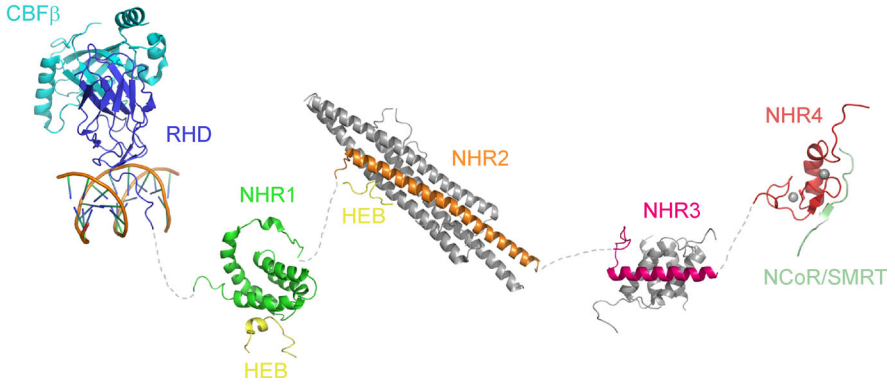

Figure 5 3D models of AML1-ETO and its complexes with binding partners. Available 3D structures of isolated AML1-ETO domains are shown by PyMOL and the structures were connected with dashed lines. RHD is colored in blue, its binding partner CBFβ in cyan, NHR1 in green, NHR2 in orange, NHR3 in hot pink, and NHR4 in red. For ETO binding partners, HEB peptides are in yellow, PKA (RIIα) in gray, and SMRT peptide in pale cyan. Note that NHR2 mediates tetramerization of AML1-ETO proteins (the other three NHR2 domains are in gray), thus bridging 4 copies of each interacting proteins. AML-ETO can also form hetero-oligomers with ETO family proteins (ETO-2, MTGR1).

residues in the LXXLL motif of PCET anchor the PCET to the α1/α4 grove (Fig. 5) (Park et al., 2009a).

Given the different binding affinity between HEB-AD1 and E2A-AD1 to NHR1, we modeled both HEB-AD1/NHR1 and E2A-AD1/NHR1 complex structures, using a longer PCET polypeptide that includes the three isoform-specific amino acids (Fig. 4A). The result showed that a Ser to Pro change in E2A PCET abolishes an H-bond interaction with a conserved Arg151 that has been previously shown to contribute to binding with HEB-AD1. In addition, the two Asp residues in the PCET motif form two salt bridges with Lys98 in HEB-AD1/NHR1 structure, but only one remains in E2A-AD1/NHR1 structure. Taken together, our docking analysis uncovered that the reduced E2A-AD1/NHR1 interaction is due to structural incompatibility with the high-affinity binding conformation (Gow et al., 2014).

The LXXLL motif in PCET is a common protein–protein interaction motif (Heery et al., 1997). In addition to its role in mediating ETO/E-protein interaction, the ETO-NHR1 (also called eTAFH) has also been shown to bind with lower affinities to NCoR, c-Myb and STAT6 via similar LXXLL motifs present in these proteins (Wei et al., 2007; Park et al., 2009a). ETO-TAFH and its homologous TAF4-TAFH (dTAFH) share similar secondary and tertiary structures. Accordingly, dTAFH uses a similar hydrophobic cleft between α1 and α4 to interact with transcription factors such as LZIP and E2F4 (Wang et al., 2007).

NHR2 domain structures

NHR2 is one of the most conserved regions in ETO family proteins. The first crystal structure of NHR2 was reported by Bushweller group (Liu et al., 2006). Four identical α-helical NHR2 polypeptides assemble into a tetrameric structure consisting of two anti-parallel-oriented dimers (Fig. 5). Site-directed mutagenesis of critical residues required for oligomerization of NHR2, but not for corepressor interactions, showed that the oligomeric potential of AML1-ETO is indispensable for its inhibition of granulocyte differentiation and enhancement of HSCs self-renewal (Liu et al., 2006).

The NHR2 of AML1-ETO also binds to E-proteins (Guo et al., 2009). Roeder and colleagues showed that NHR2/DES interaction is actually dependent on NHR2-mediated oligomerization (Sun et al., 2013). Each of the NHR2 dimers within the tetrameric structure creates a novel binding surface for the DES/N2B polypeptide (Fig. 5). As mentioned earlier, selective disruption of NHR2/N2B interaction, but not oligomerization, impaired the leukemogenic activities of both AML1-ETO and AML1-ETO9a (Sun et al., 2013).

NHR3 and NHR4 domain structures

NHR3 shares some sequence homology with A-kinase anchoring proteins (AKAP), which act as scaffold proteins to associate with cyclic AMP-dependent protein kinase (PKA) (Fukuyama et al., 2001). The structure of NHR3-PKA (RIIα) complex revealed that NHR3 folds into an amphipathic α-helix which lies on a surface formed by a dimeric PKA (RIIα) (Fig. 5). Functional studies, however, argue against an important role of this interaction in AML1-ETO's leukemogenic activities (Corpora et al., 2010; Kwok et al., 2010).

NHR4/MYND mediates AML1-ETO/ETO interactions with NCoR/SMRT (Gelmetti et al., 1998; Lutterbach et al., 1998b; Wang et al., 1998), and a second NCoR/SMRT binding site lies in ETO N-terminal part (Lutterbach et al., 1998b; Amann et al., 2001; Wei et al., 2007). The MYND/SMRT complex structure shows that MYND coordinates two zinc atoms in a manner similar to PHD and RING fingers (Gamsjaeger et al., 2007; Liu et al., 2007). MYND bound to the "PPPLI" motif present in the RD3 of SMRT to form a pair of short anti-parallel β-strands (Fig. 5). Disrupting MYND-SMRT interaction significantly altered AML1-ETO-dependent gene expression patterns but does not compromise its granulocyte differentiation inhibition function (Liu et al., 2007).

Conclusion and future directions

Since the discovery of the AML1-ETO fusion protein nearly 30 years ago, tremendous progress has been made to understand the mechanism and function of this fusion protein, including its regulation of target gene transcription, and its involvement in diverse pathways that impact on cell proliferation, differentiation, apoptosis and survival. Major advances have been made in areas of biochemical, genomic and structural studies, which provide new insights into (i) the interacting proteins of the fusion protein, (ii) the genome-wide target genes, and (iii) the atomic structures of functional domains. These studies have reinforced the idea that the fusion protein functions as a "classic" transcription factor and also established "ETO/E-protein axis" as a common axis that governs the activity of multiple leukemia fusion proteins. However, despite the major progress made in the recent years, to date, we are still far from obtaining a complete understanding of the mechanisms, function and regulation of the fusion proteins and their binding partners in normal and leukemia cells. Future studies may be directed to a further understanding of (i) the determinants that govern gene-specific activation and repression of AML1-ETO target genes; (ii) the isoform-specific function of AML1-ETO and AML1-ETO9a, (iii) the crosstalk between AML1-ETO and interacting proteins, including RUNX1 and E-proteins, (iv) the mechanism that controls the splicing of the t(8;21) fusion gene, (v) the role of the ETO family proteins in regulating the leukemia fusion proteins, and (vi) "ETO/E-protein" axis in these regulations as well as its utility as a common druggable target in leukemias. While preventing the interactions with E-

proteins is expected to inactivate t(8;21) fusion proteins in AML, enhancing the binding of ETO corepressors to E2A fusion proteins is expected to provide a new means to inactivate E2A fusion proteins in ALL. Though structural studies have uncovered new mechanisms associated with the individual domains and domain interactions, it will be important to elucidate the complete structure including protein–protein interactions that occur in the context of native proteins in living cells. Given that the AML1-ETO and E2A fusion proteins exert their functions as transcription factors, their protein–protein interactions should serve as important druggable targets for leukemia therapy.

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Compliance with ethics guidelines

Jian Li, Chun Guo, Nickolas Steinauer and Jinsong Zhang declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

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