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Biochemistry of the Mixed Lineage Leukemia 1 (MLL1) Protein and Targeted Therapies for Associated Leukemia

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1. Introduction

Mixed Lineage Leukemia constitutes a heterogeneous category of rare acute leukemias that are characterized by a mixed population of poorly differentiated lymphoid and myeloid progenitor cells. The mixed lineage leukemia (MLL1) gene, also known as HRX or ALL-1, is a frequent site of genetic rearrangements in infant acute leukemias and therapy-related malignancies (Daser and Rabbitts, 2005) and since its discovery (Djabali et al., 1992; Tkachuk et al., 1992; Ziemin-van der Poel et al., 1991), significant progress has been made in understanding its role in human biology and leukemogenesis (Liu et al., 2009; Slany, 2009). Chromosomal abnormalities involving the MLL1 gene include reciprocal chromosomal translocations, internal partial tandem duplications (PTD), and amplifications of un-rearranged MLL1 (Dou and Hess, 2008). These chromosomal aberrations are associated with mechanistically distinct gain-of-function phenotypes that may be amenable to targeted therapeutic approaches. However, progress in this area has been impeded by a lack of understanding of the molecular details by which MLL1 translocations, amplifications and PTDs contribute to leukemogenesis. To date, more than 60 MLL1 fusion partners have been described (Krivtsov and Armstrong, 2007), and detailed genetic/biochemical studies have identified several functional domains within chimeric MLL1-fusion proteins that are essential for leukemic transformation (Daser and Rabbitts, 2005; Debernardi et al., 2002; Eguchi et al., 2004; Ernst et al., 2002; Krivtsov and Armstrong, 2007; Lavau et al., 2000; Liu et al., 2009; Luo et al., 2001; Mitterbauer-Hohendanner and Mannhalter, 2004; Mueller et al., 2009; Prasad et al., 1995). Although our understanding of the molecular pathology of MLL1-associated leukemias remains incomplete, recent biochemical and structural information is contributing to an evolution of potential treatment strategies from a broadly-based chemotherapeutics approach towards therapies targeted to the underlying molecular pathogenesis of leukemia (Liedtke and Cleary, 2009). This chapter reviews recent advances in our efforts to develop novel MLL1-targeted therapies.

2. MLL1- a master epigenetic regulator with multiple roles in transcription

2.1 MLL1 in embryonic development and hematopoiesis

The MLL1 protein is a histone H3 lysine 4 (H3K4) methyltransferase that functions to maintain gene expression during development and hematopoiesis (Hess et al., 1997; Milne et al., 2002;

Yagi et al., 1998). The best studied target genes of MLL1 include the homeobox transcription factors or HOX genes, which are important for segment identity and cell fate during metazoan development (Abramovich and Humphries, 2005; Ernst et al., 2004b). Genetic studies in mice have demonstrated that the homozygous knock out of MLL1 is embryonic lethal and is associated with multiple developmental defects including neural crest patterning and hematopoietic abnormalities (Ernst et al., 2004a; Hess et al., 1997; Yagi et al., 1998; Yu et al., 1995). Notably, the expression levels of several HOX genes including HOXA4, HOXA7, HOXA9, and HOXA10 are decreased in MLL1^{-/-} mice and are associated with defects in fetal liver hematopoiesis (Hanson et al., 1999; Yagi et al., 1998). MLL1^{+/-} mice are not embryonic lethal but are anemic and exhibit homeotic developmental defects that are related to posterior shifts in HOX gene expression patterns (Yagi et al., 1998; Yu et al., 1995). Likewise, MLL1 is also required for adult hematopoiesis and stem cell self-renewal. In MLL1 conditional knockout mice fetal hematopoiesis is unaltered; however, adult mice exhibit anemia, a significant reduction in the number of bone marrow hematopoietic stem cell progenitors, and poor survival rates (Gan et al., 2010). In addition, it has been demonstrated that MLL1 plays a crucial role in self-renewal in cultured fetal liver and adult bone marrow stem cells (Jude et al., 2007; McMahon et al., 2007). These studies suggest that MLL1 orchestrates its biological functions at least in part through the regulation of HOX genes. Indeed, HOX dysregulation is a common phenotype that underlies the pathogenesis of acute leukemias associated with alterations in the MLL1 gene (Armstrong et al., 2002; Ayton and Cleary, 2003; Dorrance et al., 2006; Ferrando et al., 2003; Liu et al., 2009). However, MLL1 is also required for the regulation of cell cycle dependent genes such as: cyclins A, B, and E (Takeda et al., 2006) and CDK inhibitors p16^{Ink4a}, p18, p27 (Milne et al., 2005; Takeda et al., 2006); E2F family of transcription factors (E2F2, E2F4 and E2F6) (Takeda et al., 2006) as well as the transcription factor GATA3, which plays an essential role in specifying lymphoid subtype (Yamashita et al., 2006). In addition, MLL1 regulates expression of several genes involved in organogenesis and differentiation (Ansari and Mandal, 2010; Scharf et al., 2007). Therefore, MLL1 is a master regulator that is critical for many gene expression programs required for normal development, hematopoiesis and the cell cycle.

2.2 MLL1 regulates the degree of H3K4 methylation and transcription

In eukaryotes, DNA is condensed into highly ordered structures known as chromatin- the structure of which is dynamically altered according to the needs of the cell. The basic repeating unit of chromatin is the nucleosome, which is composed of ~146 base pairs of DNA wrapped around an octameric disc of histone proteins containing two copies each of histones H2A, H2B, H3 and H4 (Luger and Hansen, 2005). Cellular processes that require access to DNA often use enzymes that dynamically regulate the structure of chromatin either through recruitment of adaptor proteins or additional enzymatic machineries that alter the positioning of nucleosomes on DNA (Cosgrove and Wolberger, 2005). One such enzymatic activity is the methylation of lysine 4 of histone H3 (H3K4), an evolutionarily conserved epigenetic mark predominantly associated with transcriptional activation in eukaryotes (Bernstein et al., 2002; Boggs et al., 2002; Litt et al., 2001; Noma and Grewal, 2002; Strahl et al., 1999). The epsilon amino group of lysine 4 can be mono-, di-, or trimethylated, with each modification correlating with distinct transcriptional outcomes (Bernstein et al., 2005; Ng et al., 2003; Pokholok et al., 2005; Santos-Rosa et al., 2002; Schneider et al., 2004; Schubeler et al., 2004). For example, genome-wide chromatin immunoprecipitation studies

have demonstrated that high levels of H3K4 trimethylation are present within the 5' regions of actively transcribed genes (Pokholok et al., 2005; Santos-Rosa et al., 2002; Schneider et al., 2004; Schubeler et al., 2004). It has been demonstrated that H3K4 trimethylation functions to recruit ATP dependent nucleosome remodeling enzymes that increase promoter DNA accessibility by sliding or displacing nucleosomes (Pray-Grant et al., 2005; Santos-Rosa et al., 2003; Wysocka et al., 2006). H3K4 dimethylation is spread more evenly across the coding regions of genes and is thought to be associated with a transcriptionally "poised" state of chromatin (Bernstein et al., 2002; Schneider et al., 2004; Schubeler et al., 2004). In contrast, H3K4 monomethylation is enriched at the 3' ends of the genes and distal enhancer sequences, and is associated with ribosomal DNA (rDNA) and telomeric silencing (Bernstein et al., 2002; Heintzman et al., 2007; Pokholok et al., 2005; Santos-Rosa et al., 2002; Schneider et al., 2004; Schubeler et al., 2004) (Nislow, Ray et al. 1997; Briggs, Bryk et al. 2001; Schneider, Wood et al. 2005; van Dijk, Marley et al. 2005). These studies suggest that the degree of H3K4 methylation is a highly regulated process. Indeed eukaryotes have evolved a number of highly conserved enzymes whose function appears to precisely regulate the degree of H3K4 methylation.

H3K4 methylation is mainly deposited by a group of enzymes that share an evolutionarily conserved SET (SuVar, E(z), Trithorax) domain (Dillon et al., 2005), although a new H3K4 methyltransferase lacking a SET domain has recently been reported (Patel et al., 2009; Patel et al., 2011). While there are several SET domain enzymes that differ with regard to their substrate specificity (Dillon et al., 2005; Qian and Zhou, 2006), members of the SET1 family share the properties that they all methylate H3K4, and all interact with an evolutionarily conserved core group of proteins that function to regulate the degree of H3K4 methylation. MLL1 belongs to the SET1 family of histone methyltransferases and evidence suggests that its transcriptional co-activator function is mediated in part by the enzymatic activity of its SET domain (Dillon et al., 2005; Milne et al., 2002). For example, homozygous deletion of the MLL1 SET domain in mice, while not embryonic lethal, exhibits skeletal defects and altered expression of several HOX genes that partially phenocopy the heterozygous knockout of the whole MLL1 gene (Terranova et al., 2006). These changes are correlated with decreased levels of mono- and dimethylation of H3K4 and deregulated DNA methylation patterns at several HOX gene promoters (Terranova et al., 2006).

MLL1 functions within a large macromolecular complex with more than 30 subunits that regulate the degree of H3K4 methylation and MLL1's target gene specificity (Cosgrove and Patel, 2010). While subunit composition of different SET1 family members varies to some degree, each SET1 family member interacts with a conserved core group of proteins that include WD-40 repeat protein-5 (WDR5), Retinoblastoma binding protein-5 (RbBP5), Absent small homeotic 2-like protein (Ash2L) and Dumpy30 (Dpy-30) (Cho et al., 2007; Dou et al., 2006; Lee et al., 2007). WDR5, RbBP5, Ash2L and Dpy-30 form an independent complex called WRAD that possesses an intrinsic histone methyltransferase activity on its own (Patel et al., 2009; Patel et al., 2011). When WRAD interacts with MLL1, it forms what is known as the MLL1 core complex, which is required for mono- and dimethylation of H3K4 (Dou et al., 2006; Patel et al., 2009). These studies have led to a model in which H3K4 methylation is sequentially catalyzed by a complex that contains multiple distinct active sites for the addition of each methyl group (Patel et al., 2009).

2.3 Mechanism of multiple lysine methylation catalyzed by MLL1 core complex

Previously it was thought that mono-, di-, and trimethylation of H3K4 could be attributed to the SET domain dependent methyltransferase activity of MLL1 alone and that the WRAD

complex functions merely as an allosteric regulator of MLL1 (Cheng et al., 2005; Collins et al., 2005; Dou et al., 2006; Han et al., 2006; Ruthenburg et al., 2006; Southall et al., 2009; Steward et al., 2006; Takahashi et al., 2009). However, since it has more recently been demonstrated that the WRAD complex dimethylates H3K4 in a manner that is independent of the enzymatic activity of the MLL1 SET domain (Patel et al., 2009), the allosteric model needs to be revised. Indeed, *in vitro* methylation assays demonstrate that the isolated MLL1 SET domain is predominantly a monomethyltransferase, which can be attributed to the presence of a conserved tyrosine residue in the SET domain active site (Patel et al., 2009). Furthermore, loss of the WDR5, RbBP5 or Ash2L subunits of WRAD results in the loss of di- and trimethylation of H3K4 both *in vivo* and *in vitro* without significant changes in H3K4 monomethylation (Dou et al., 2006; Patel et al., 2009; Patel et al., 2008b; Wysocka et al., 2005). These results are consistent with a sequential mechanism whereby the MLL1 SET domain catalyzes H3K4 monomethylation and the WRAD enzyme catalyzes H3K4 dimethylation within the MLL1 core complex. However, WRAD lacks the ability to dimethylate H3K4 without MLL1, suggesting that MLL1 amino acid sequences, distinct from the MLL1 SET domain active site, contributes to the WRAD active site within the MLL1 core complex. That a complex between WRAD and MLL1 is required for H3K4 dimethylation is supported by the demonstration that amino acid substitutions that disrupt the interaction between MLL1 and WRAD also disrupt the H3K4 dimethylation activity of the MLL1 core complex (Patel et al., 2008b). Therefore, the completely assembled MLL1 core complex is required for efficient H3K4 dimethylation and for nucleosome methylation (Patel et al., 2011). The existence of a sequential mechanism utilizing several active sites for multiple lysine methylation suggests that the degree of H3K4 methylation is more highly regulated than previously appreciated.

2.4 WRAD components are associated with development and oncogenesis

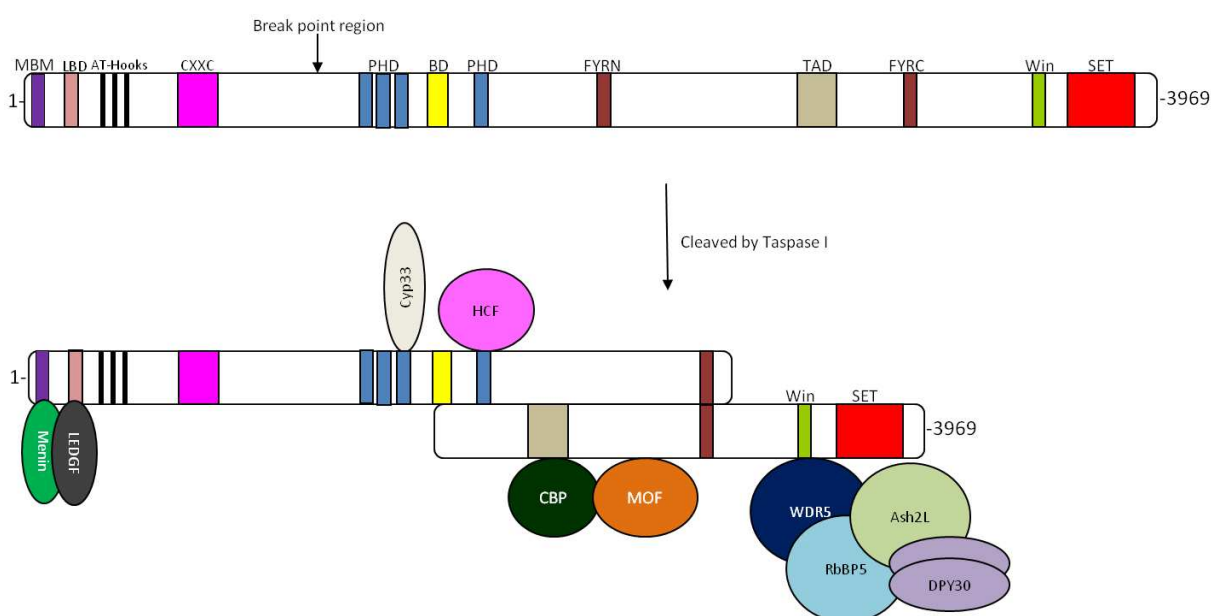
One of the common features of the greater than 60-MLL fusion proteins associated with leukemias is that in most cases they lose the amino acid sequences required for interaction with WRAD. This raises questions about WRAD's role in MLL1 associated oncogenesis. WRAD subunits are conserved within SET1 family complexes ranging from yeast to humans (Cho et al., 2007; Dou et al., 2006; Lee and Skalnik, 2005; Lee et al., 2007; Steward et al., 2006) and have been shown to play essential roles in cellular differentiation (Gori and Demay, 2005; Gori et al., 2005; Zhu et al., 2008), development (Adamson and Shearn, 1996; Wysocka et al., 2005), dosage compensation (Hsu et al., 1995; Hsu and Meyer, 1994), embryogenesis (Stoller et al.), and transcription (Tan et al., 2008). However, the WRAD enzyme lacks sequence homology to known methyltransferase folds and as a result relatively little is understood about its methyltransferase activity and the role it might play in oncogenesis. WRAD's potential role in oncogenesis is supported by the observation that the Ash2L component of WRAD is overexpressed at the protein level in many human tumors, and that knockdown of Ash2L inhibits tumor cell proliferation (Luscher-Firzlauff et al., 2008). In addition, the RbBP5 component of WRAD has been shown to be amplified in several glioblastomas, suggesting that it may be a novel oncogene (Bralten et al., 2010). Further studies will be required to better understand WRAD's role in MLL1 associated leukemogenesis and other cancers.

In contrast, the MLL1 component of the MLL1 core complex is more extensively characterized and several functional domains implicated in transcriptional regulation have been identified (for recent reviews refer to Ansari and Mandal, 2010; Cosgrove and Patel, 2010). Furthermore, MLL1 is a frequent site of chromosomal alterations that sometimes

disrupt the functions of these domains. The next subsection summarizes recent genetic, biochemical and structural studies of the functional domains that regulate MLL1's gene targeting and H3K4 methyltransferase activity.

2.5 MLL1 functional domains implicated in transcriptional regulation

The MLL1 gene encodes a large protein of 3,969 amino acid residues and contains several functional domains including: menin binding motif (MBM), Lens epithelium derived growth factor (LEDGF) binding domain (LBD), DNA-binding AT hooks, a cysteine-rich CXXC DNA binding motif, plant homeodomain (PHD) fingers, a bromo domain (BD), a transactivation domain (TAD), a WDR5 interaction (Win) motif, and a C-terminal histone methyltransferase SET domain (Figure 1) (Cosgrove and Patel, 2010). The full-length MLL1 protein, synthesized as a single transcript, is cleaved by taspase I into MLL1-N (320 kDa) and MLL1-C (180 kDa) fragments, which then re-associate through the FYRN and FYRC motifs to form the functional MLL1 complex in vivo (Figure 1) (Hsieh et al., 2003a; Hsieh et al., 2003b; Yokoyama et al., 2002). The mature MLL1 protein assembles into macromolecular complexes with several regulatory proteins that are essential for MLL1's transcriptional co-activator properties. Biochemical and genetic studies have identified several direct and



Schematic representation showing the functional domains present in the MLL1 protein. Menin binding motif MBM (purple), LEDGF binding domain or LBD (light red), DNA binding AT-hooks (black), zinc finger containing CXXC motifs (pink), plant homeodomain (PHD) fingers (blue), bromodomain (BD) (yellow), phenylalanine-tyrosine rich regions (FYR) (brown), WDR5 interaction (*Win*) motif (light green), and the histone methyltransferase SET domain (red) are highlighted. a) The full-length MLL1 protein (3969 amino acids) is cleaved by Taspase 1 into MLL-N (300 kDa) and MLL-C (180 kDa) fragments that then re-associate through FYRN and FYRC motifs to form a stable complex. This mature MLL1 protein then assembles into a macromolecular complex with a number of proteins including- menin (green); lens epithelium derived growth factor or LEDGF (dark grey); nuclear cyclophilin (Cyp33) (light grey); host cell factor (HCF) (light pink); CREB binding protein (CBP) (dark green); histone acetyltransferase MOF (orange); WD-40 repeat containing protein-5 (WDR5) (dark blue); retinoblastoma binding protein-5 (RbBP5) (cyan); absent, small, homeotic disc-2 like (Ash2L) (olive green); and DPY-30 (light purple).

Fig. 1. Domain architecture of human MLL1.

indirect interaction partners for MLL1 that include; the menin tumor suppressor protein (Hughes et al., 2004; Yokoyama et al., 2004); cell cycle regulators such as E2Fs and HCF-1 (Tyagi et al., 2007; Yokoyama et al., 2004); polycomb group proteins, BMI-1 and HPC-2 (Xia et al., 2003); histone deacetylases (Nakamura et al., 2002; Xia et al., 2003); nuclear cyclophilin, Cyp33 (Xia et al., 2003); acetyltransferases such as p300, CBP and MOF (Dou et al., 2005; Ernst et al., 2001); chromatin remodeling factors, INI1/SNF5 (Rozenblatt-Rosen et al., 1998); and WDR5/RbBP5/Ash2L and DPY30, which are core components of SET1 family methyltransferases (Dou et al., 2006). In addition, a recent paper describes the identification of a gene internal promoter that transcribes the C-terminal half of MLL1 (Scharf et al., 2007), the function of which is not known. Interestingly, the gene internal promoter coincides with an MLL1 breakpoint region, suggesting that the pathogenesis of MLL1 translocation induced leukemia could be due in part to the loss of this N-terminally truncated form of MLL1 when the breakpoint is 5' to the gene internal promoter. However, in cases where the MLL1 breakpoint is 3' to the gene internal promoter, it could result in aberrant expression of the fusion protein (Scharf et al., 2007). Indeed, it has been noted that the common MLL1 translocation partners AF4, AF6, and ENL have potential AUG start codons in positions where they could be transcribed within the context of the translocated MLL1 gene (Scharf et al., 2007).

These studies suggest that the transcriptional activator properties of MLL1 are mediated through multiple functional domains through protein-protein and protein-DNA interactions. Many of these interactions are retained in leukemogenic MLL1s, some participating in gain-of function phenotypes, making them candidates for molecular targeted therapy. We review progress in this area in sections 3-5. In section 2, we describe in more detail our current understanding of the role of MLL1 alterations in hematopoietic malignancies.

3. MLL1-A key player in hematologic malignancies

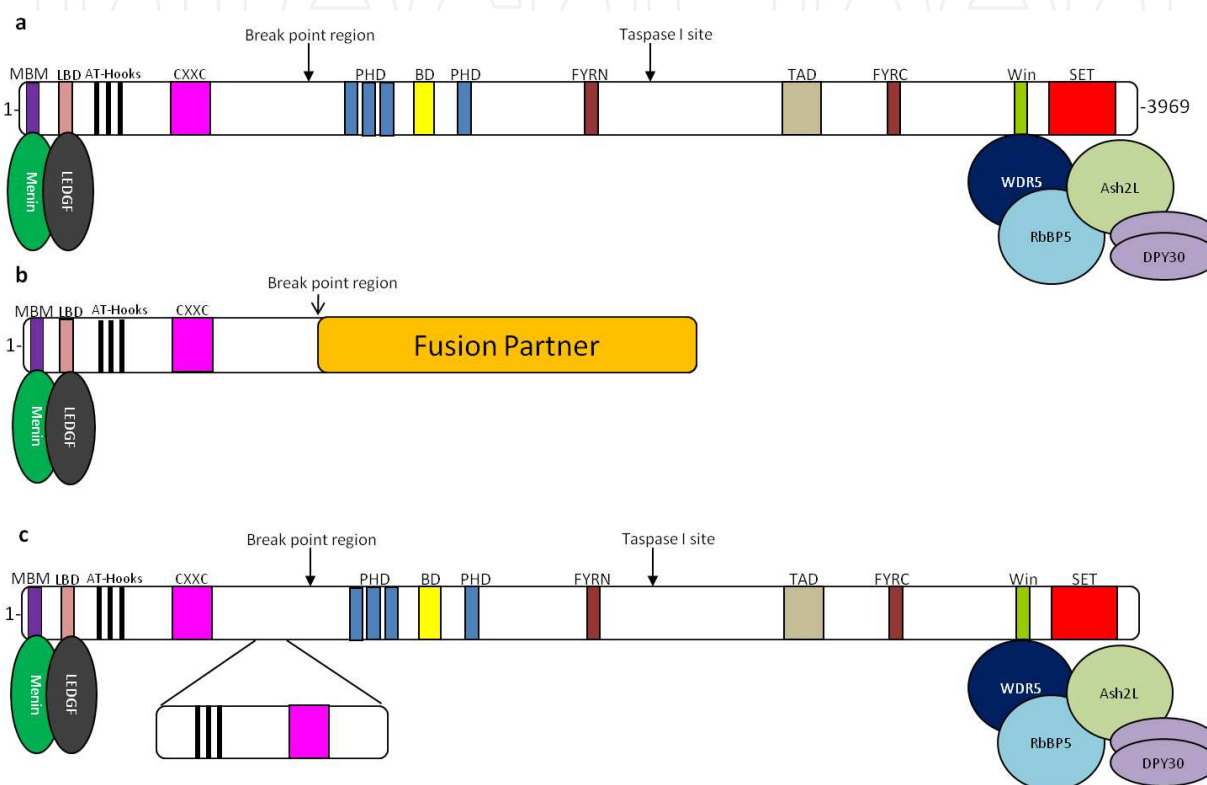
3.1 Acute myeloid and lymphoblastic leukemia with 11q23 abnormalities

3.1.1 Incidence and clinical significance of 11q23 chromosomal translocations

The MLL1 gene located at chromosome 11, band q23, is frequently involved in reciprocal translocations found in several cases of acute myeloid (AML) and acute lymphoblastic leukemia (ALL) (Djabali et al., 1992; Gu et al., 1992; Ziemin-van der Poel et al., 1991) and identify a patient sub-population with a poor prognosis (Daser and Rabbitts, 2005). Recurrent MLL1 translocations account for >70% of infant acute leukemias (both ALL and AML) and are also observed in approximately 10% of de novo AML in adults (Krivtsov and Armstrong, 2007), and in therapy-related leukemias that develop in patients treated with topoisomerase II inhibitors (Bigoni et al., 1999; Felix et al., 1995; Krivtsov and Armstrong, 2007). Chromosomal translocations fuse the N-terminal part (~1400 amino acids) of the MLL1 protein in-frame to one of more than 60 partner proteins that range from nuclear factors to cytoplasmic proteins (Daser and Rabbitts, 2005; Huret et al., 2001; Schoch et al., 2003).

The five most common MLL1 translocations include: MLL1-AF4 or t(4;11)(q21;q23); MLL1-ENL or t(11;19)(q23;p13.3); MLL1-AF9 or t(9;11)(p23;q23), MLL1-AF10 or t(10;11)(p12;q23), and MLL1-AF6 or t(6;11)(q27;q23) and account for greater than 80% of MLL1-rearranged leukemias (Burmeister et al., 2009; Meyer et al., 2009; Meyer et al., 2006; Slany, 2009). In addition, chimeric MLL1-fusions involving ELL, EEN, GAS7, AF1p, AFx, Septins, and histone acetyltransferases CBP/p300 have also been reported (Bernard et al., 1994; Dobson et al., 2000; Hall and Russell, 2004; Ida et al., 1997; Krivtsov and Armstrong,

2007; Meyer et al., 2009; Schichman et al., 1995; So et al., 2003; Taki et al., 1997; Tkachuk et al., 1992; Wang et al., 2005). The translocation partners identified to date are diverse and do not share any biochemical function or structural motifs. However, all known MLL1 fusion proteins share the property that the N-terminal portion containing the AT hooks and CxxC domains of MLL1 are retained, suggesting the preservation of DNA binding activity (Ayton et al., 2004; Macrini et al., 2003). In contrast, the taspase cleavage site, gene internal promoter, TAD domain, PHD fingers, *Win* motif and the SET domain are lost (Figure 2) (Liu et al., 2009).



The putative protein products generated by the two most common chromosomal alterations associated with the MLL1 gene are indicated along with wild-type MLL1: a) wild-type MLL1, b) reciprocal chromosomal translocations involving MLL1, and c) partial tandem duplications (PTDs) in MLL1. The various functional domains are color coded as in Figure 1. Chromosomal translocations fuse the N-terminal ~1400 amino acids of MLL1 in-frame to one of over 60 different fusion partners (indicated in orange in b). PTDs have a duplicated N-terminus (AT-hooks and CXXC motifs) in addition to all the functional domains present in wild-type MLL1.

Fig. 2. Schematic representation of the most common genetic alterations associated with the MLL1 gene.

3.1.2 Pathogenesis of acute leukemias with MLL1-translocations

While it is expected that the loss of SET domain in MLL1 translocations would result in decreased H3K4 methylation and Hox gene expression, genetic studies have revealed that the individual fusion partners possess transcriptional activator properties and are indispensable for leukemogenesis (Chen et al., 2006a; Dobson et al., 1999; Dobson et al., 2000; Wang et al., 2005). Given the complexity of different translocation partners, MLL1-fusions may activate a common leukemia-associated gene expression program through multiple mechanisms. Indeed, AF4, AF5, AF9, ENL, ELL and AF10 proteins are all

implicated in transcriptional elongation via association with the EAF complex, pTEFb kinase, and hDOT1 mediated methylation of H3K79 (Bitoun et al., 2007; Luo et al., 2001; Mueller et al., 2007; Mueller et al., 2009; Okada et al., 2005; Simone et al., 2001). Another mechanism could involve transcriptional activation via increased or aberrant histone acetylation (MLL-CBP/p300) (Lavau et al., 2000; Sobulo et al., 1997), protein arginine methyltransferase-1 (PRMT1) association (MLL-EEN) (Cheung et al., 2007), SWI-SNF chromatin-remodeling complex recruitment (MLL-ENL, -AF9, -AF10) (Debernardi et al., 2002; Nie et al., 2003; Schreiner et al., 1999), and self-association or dimerization of the N-terminal part of MLL1 (MLL-GAS7, -AF1p, -beta-galactosidase, -gephyrin, -SEPT6) (Dobson et al., 2000; Eguchi et al., 2004; Martin et al., 2003; So et al., 2003).

Regardless of the mechanism, aberrant expression of MLL1 target genes are a common feature of MLL1-rearrangements examined to date. For example, HOXA7, HOXA9, and the HOX cofactor MEIS1 are consistently over expressed in human leukemias with MLL1-translocations (Armstrong et al., 2002; Ayton and Cleary, 2003; Rozovskaia et al., 2001; Yeoh et al., 2002; Zeisig et al., 2004) and act, at least partially, through the activation of the proto-oncogene c-Myb (Hess et al., 2006). Furthermore, retroviral co-transduction studies in mice have demonstrated that HOXA9 and MEIS1 expression immortalizes hematopoietic progenitors in vitro and rapidly accelerates leukemia development (Kroon et al., 1998). In addition, MLL1-fusion proteins fail to transform bone marrow cells in which HOXA7 and HOXA9 expression are genetically ablated (Ayton and Cleary, 2003). Similarly, it has been demonstrated that expression of HOXA9 and MEIS1 can replace the leukemogenic activity of MLL1-ENL (Zeisig et al., 2004). Collectively, these results suggest that HOXA9 dysregulation is an important factor in some MLL1-fusion induced leukemias (Ayton and Cleary, 2003). However, not all leukemogenic fusions result in HOXA9 dysregulation. For example, MLL1-GAS7 and MLL1-AF9 fusions were shown to transform bone marrow cells or mice that do not express HOXA9 (Kumar et al., 2004; So et al., 2004).

However, in addition to dysregulation of HOX genes, other signaling pathways are perturbed by MLL-translocations and may contribute to leukemogenesis. For example, transcriptional deregulation of FMS-like tyrosine kinase 3 (FLT3), glycogen synthase kinase 3 (GSK3), heat shock protein-90 (HSP-90), myeloid cell leukemia sequence-1 (MCL-1), and components of the RAS pathway have been implicated in MLL1-induced leukemogenesis (Armstrong et al., 2003; Brown et al., 2005; Carnicer et al., 2004; Liang et al., 2006; Stubbs et al., 2008; Wang et al., 2008; Yao et al., 2005; Yasui et al., 2005; Yocum et al., 2006). MLL1's role as a master regulator of gene expression significantly complicates understanding its role in MLL1 associated leukemogenesis.

The master regulatory role of MLL1 in transcriptional control has implications that affect our normal understanding of malignancy. For example, it has been suggested that second-hit mutations are required to initiate the full leukemia phenotype (Dobson et al., 2000). Indeed, recent studies have identified mutations in p53, ATM, Ras, and FLT3 genes in MLL1 leukemia patients (Felix et al., 1998; Mahgoub et al., 1998; Oguchi et al., 2003; Taketani et al., 2004). However, because of MLL1's role in epigenetic gene control, second hit mutations could also arise in the form of epigenetic mutations that result in silencing of tumor suppressors genes without changes in their DNA sequence. For example, it has been demonstrated that the FHIT tumor suppressor gene is epigenetically silenced in human primary tumor cells and tumor cell lines with MLL1-translocations (Stam et al., 2006). These data suggest that epigenetic alterations may be just as important as genetic mutations in sources of so called "2nd hit" mutations that underlie the pathogenesis of leukemia. Recent advances in deep sequencing technologies such as RNA-SEQ and CHIP-SEQ will likely allow us to better distinguish genetic versus epigenetic aberrations in future studies.

3.2 Partial tandem duplications: A cytogenetically normal rearrangement in MLL1

3.2.1 Clinical significance of MLL1 partial tandem duplications (MLL1-PTDs)

The second common MLL1-rearrangement, internal partial tandem duplication (MLL1-PTD) was first observed in de novo AML patients with a normal karyotype or trisomy 11 (Caligiuri et al., 1994). MLL1-PTDs are found in 4-7% of the cases of AML and present a cytogenetically normal rearrangement that is associated with poor prognosis (Caligiuri et al., 1998; Dohner et al., 2002; Schichman et al., 1994; Schichman et al., 1995). MLL1-PTDs result from an in frame fusion of exons 11-5 or 12-5 upstream of exon 5, partially duplicating sequences in the 5' end of MLL1 (Caligiuri et al., 1994; Quentmeier et al., 2003; Schichman et al., 1994). The protein product of MLL1-PTDs has a duplicated N-terminus that contains an additional AT-hook and CXXC domain while essentially retaining all the conserved domains in wild-type MLL1 (Quentmeier et al., 2003; Schichman et al., 1994). In contrast to the variety of MLL1-fusions that delete the MLL1 C-terminus, MLL1-PTDs retain the 3'-portion of the gene that encodes the SET domain methyltransferase motif (Figure 2). Recent data suggests that the enzymatic activity of the MLL-PTD SET domain participates in a gain-of function phenotype in AML.

3.2.2 Mechanism of leukemic transformation by MLL1-PTDs

The molecular mechanisms that underlie MLL1-PTD transformation in AML are currently unknown. The crucial alteration is the duplication of AT hooks and CXXC DNA binding motifs, which may alter target gene specificity. It has been suggested that duplication of these segments may also mimic dimerization observed in several MLL1 chimeric fusion proteins (Martin et al., 2003). Indeed, it has been demonstrated that a synthetic MLL1 N-terminal construct containing duplicated AT-hook and CXXC domains possesses potent transactivation activity in luciferase reporter assays (Martin et al., 2003). However, comparison of genome-wide gene expression data shows that MLL1-PTD primary cells have gene expression patterns that are distinct from that of cells bearing MLL1 chimeric fusions suggesting that the mechanism underlying transformation is distinct (Ross et al., 2004). Alternatively, it has been suggested that the additional amino acid sequences in MLL1-PTD might adopt a conformation that potentially interferes with the normal function of MLL1 by distancing the regulatory domains from its target site (Dou and Hess, 2008).

There is a growing body of evidence that suggests that epigenetic alterations underlie the pathogenesis of MLL1-PTDs. For example, it has been demonstrated that knock-in mice bearing MLL1^{PTD/WT} exhibit increased expression of HOXA7, HOXA9, and HOXA10 that is associated with increased H3K4 methylation and H3/H4 acetylation within these promoters (Dorrance et al., 2006). Similar gene expression and H3K4 dimethylation changes were seen in the presence and absence of the wild type MLL1 allele in primary MLL1-PTD mouse fetal liver cells, suggesting that MLL1-PTD behaves as a dominant gain-of-function mutation (Dorrance et al., 2008). This phenotype may also be due to other epigenetic alterations. For example, Whitman and colleagues (2005) have demonstrated that the wild type MLL1 allele in MLL1^{PTD/WT} AML cells is silenced in a manner that is associated with hypoacetylation of histones H3 and H4 (Whitman et al., 2005). Treatment of MLL1-PTD cells with histone deacetylase inhibitors partially reactivates wild type MLL1 expression and reduces AML blast colony forming units (Whitman et al., 2008). In addition, it has been demonstrated that the SLC5A8 tumor suppressor gene is silenced in MLL-PTD cells in a manner that is associated with increased DNA methylation in its promoter, a phenotype that is partially

reversed with DNA methyltransferase inhibitors (Whitman et al., 2008). SLC5A8 encodes a membrane monocarboxylate transporter that regulates intracellular concentrations of histone deacetylase inhibitors, such as butyrate and pyruvate (Ganapathy et al., 2005; Gupta et al., 2006). Together, these observations suggest that targeting MLL1-PTD may have therapeutic value in the treatment of AML (Whitman et al., 2005). Indeed, it has been demonstrated that down regulation of MLL1-PTD using antisense oligodeoxynucleotides (aODNs) in primary human MLL-PTD AMLs results in reactivation of the wild type MLL1 gene, reduced AML blast-derived colony forming units, and increased sensitivity to cell death (Whitman et al., 2008).

3.3 Acute myeloid leukemia with amplifications in MLL1

Amplifications of the MLL1 gene, including trisomy 11 and intrachromosomal amplifications, are found with less frequency in AML and other myelodysplastic syndromes and are associated with a complex karyotype and poor prognosis (Allen et al., 1998; Ariyama et al., 1998; Avet-Loiseau et al., 1999; Cuthbert et al., 1999; Herry et al., 2006; Poppe et al., 2004; Streubel et al., 2000). Amplifications involving MLL1 result in up-regulation of several HOX genes including, HOXA7, HOXA9, and MEIS1 (Herry et al., 2006; Poppe et al., 2004). These studies suggest that MLL1-amplifications contribute to leukemogenesis through mechanisms that share some features with that of MLL1-fusions and MLL1-PTDs. In addition, gene expression analyses have identified other proteins that are up-regulated in AML with 11q23 amplifications including cell surface receptors PROM1, ADAM10, and NKG2D, and the inosine triphosphatase (ITPA) (Poppe et al., 2004). These data suggest that MLL1 amplifications are associated with a gain-of-function phenotype that may be responsive to targeted therapy (Poppe et al., 2004).

In spite of these advances in our understanding of the pathogenesis of MLL1-linked leukemias, identification of inhibitors that specifically target MLL1 or MLL1-chimeric fusion proteins has so far proven elusive. Although in recent years the introduction of broadly based chemotherapeutic interventions such as all-trans retinoic acid, cytosine arabinoside, histone deacetylase and DNA methyltransferase inhibitors has increased the survival rates in some leukemia patients (Altucci et al., 2005; Downing, 2008; Liedtke and Cleary, 2009), molecular therapies that target MLL1 are still lacking. Recent advances in our understanding of the protein-protein interactions involving MLL1 suggest several novel therapeutic strategies for targeted inhibition of MLL1 or MLL1 chimeric fusion activity in leukemic cells. These advances are reviewed in sections 3-6 (and are summarized in Figure 8).

4. Targeting MLL1-Menin interaction as a therapeutic strategy to treat Mixed Lineage Leukemia

4.1 Menin is a common component of wild-type and mutant MLL1 complexes

Despite the growing evidence demonstrating a role for HOX genes in hematopoiesis, it is unclear how the array of mutations involving the MLL1 gene all contribute to altered HOX gene expression in acute leukemias. While there is little that is common among the different types of MLL1 aberrations, all types of MLL1 gene mutations including: chromosomal translocations, internal partial tandem duplications, and gene amplifications, retain the N-terminus of MLL1 (Daser and Rabbitts, 2005; Hess, 2004; Mitterbauer-Hohendanner and Mannhalter, 2004). These ~1400 N-terminal amino acids contain the DNA binding AT-hooks

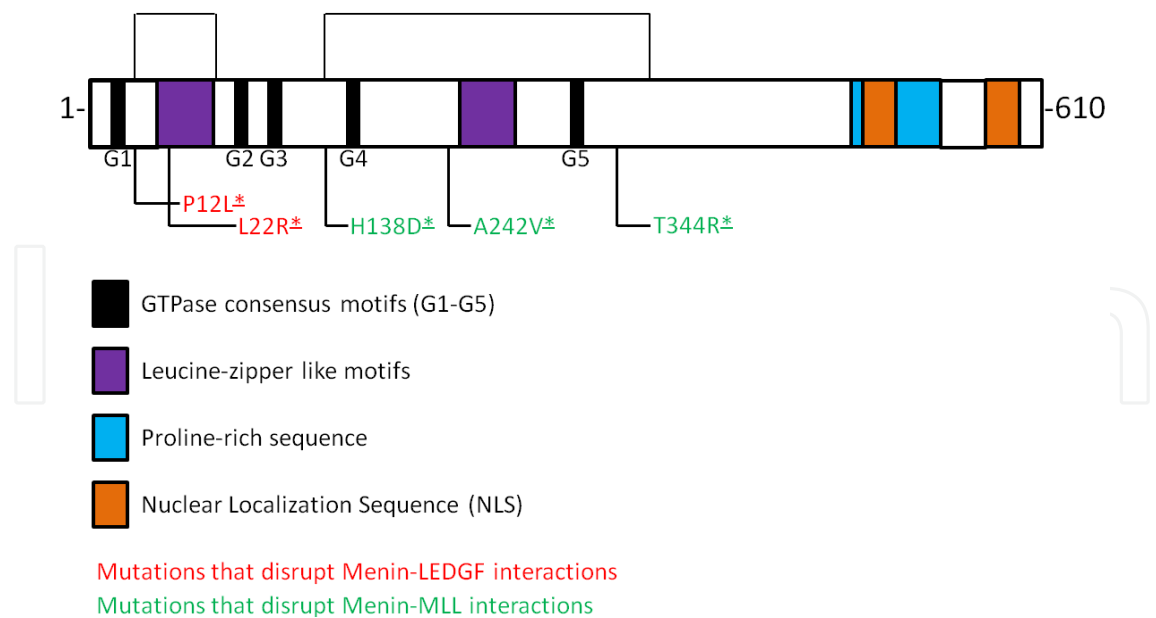
and CXXC domains as well as the binding site for nuclear proteins menin (called the Menin Binding Motif or MBM) and the LEDGF binding domain (LBD) (Figures 1 and 2). Menin, which directly binds wild-type MLL1 and MLL1-oncogenic fusion proteins, is an essential co-factor for the maintenance of normal hematopoiesis and the leukemogenic activity of MLL1-associated translocations (Chen et al., 2006b; Hughes et al., 2004; Yokoyama et al., 2005; Yokoyama et al., 2004). The importance of menin in the pathogenesis of MLL1-related leukemia and progress on the development of inhibitors that target the MLL1-menin interaction is reviewed in this section.

4.2 MEN1 tumorigenesis

Menin is the product of MEN1 gene located at chromosome band 11q13. Menin functions as a tumor suppressor protein that is mutated in patients with an inherited syndrome called Multiple Endocrine Neoplasia 1 (MEN1) (Chandrasekharappa et al., 1997; Chandrasekharappa and Teh, 2001; Larsson et al., 1988). To date, more than 400 nonsense and frame-shift mutations have been reported in MEN1 patients often developing parathyroid, pancreatic or pituitary tumors after the loss of the wild-type MEN1 allele (Dong et al., 1997; Larsson et al., 1988; Lemmens et al., 1997; Thakker, 2001). Homozygous knockout of MEN1 (-/-) is embryonic lethal in mice, which die at the mid-gestation period with profound defects in liver, heart and the neural tube (Bertolino et al., 2003a; Crabtree et al., 2001; Stewart et al., 1998). Heterozygous knockout mice are viable until the adult stages, but develop tumors similar to human MEN1 syndrome in pancreatic islets, parathyroid, anterior pituitary, adrenal cortex and adrenal medulla (Bertolino et al., 2003b; Crabtree et al., 2001). Although the loss of menin results in tumors of the endocrine lineage, the MEN1 gene is ubiquitously expressed in most adult tissues and at all developmental stages (Chandrasekharappa and Teh, 2001, 2003). In spite of the increasing evidence that highlights a role for menin in MEN1 tumors, the basic biology of menin dependent tumor suppression is unclear.

4.3 Menin homology and conserved domain architecture

Menin is highly conserved among vertebrates including, humans, mouse, rat, and zebrafish. However, menin orthologs have not been identified in budding yeast *Saccharomyces cerevisiae* or in the nematode *Caenorhabditis elegans* (Stewart et al., 1998) (Guru et al., 1999; Guru et al., 2001; Khodaei et al., 1999; Manickam et al., 2000). Strikingly, several of the disease-associated MEN1 mutations occur at conserved amino acid positions, highlighting a crucial role for menin in regulating cell proliferation in higher eukaryotes (Chandrasekharappa and Teh, 2003; Poisson et al., 2003). Menin is a novel nuclear protein of ~610 amino acids and does not share significant sequence homology to any other known proteins (Chandrasekharappa et al., 1997; Guru et al., 1998; Poisson et al., 2003). However, extensive analysis of sequence alignments among menin homologues has revealed several domains with putative roles in nuclear targeting and transcriptional regulation. Conserved domain search using the human menin amino acid sequence identified putative domains such as: consensus GTPase-like motifs (G1-G5), two leucine-zipper motifs, a proline-rich region and two nuclear localization signals (NLS) (Balogh et al., 2006; Chandrasekharappa and Teh, 2003; Poisson et al., 2003) (Figure 3). However, it is unclear as to how these conserved motifs contribute to the tumor suppressor function of menin.



Schematic representation of the conserved regions in human menin with the various functional domains indicated in different colors: GTPase consensus-motifs G1-G5 (black), leucine-zipper-like motifs (purple), proline-rich sequence (blue), and nuclear localization signal (NLS) (orange). Naturally occurring MEN1 mutations that disrupt its interaction with MLL1 (green) and LEDGF (red) are indicated below.

Fig. 3. Domain architecture of human menin showing conserved domains

4.4 Menin molecular interaction network

The precise biochemical function of menin has so far proven elusive due to the lack of any known functional domains in menin. However, it has been suggested that the transcriptional regulatory properties of menin are regulated by protein-protein interactions (Balogh et al., 2006; Jin et al.; Poisson et al., 2003; Yokoyama and Cleary, 2008; Yokoyama et al., 2004). Recent biochemical efforts have been undertaken to elucidate the molecular pathways that underlie menin-dependent transcriptional regulation (Balogh et al., 2006). Using yeast two-hybrid, GST pull-down and co-immunoprecipitation assays, menin was shown to interact with a cohort of proteins that are involved in cell cycle regulation, DNA replication and repair, genome stability, endocrine metabolism, bone morphogenesis and hematopoiesis (Balogh et al., 2006; Chandrasekharappa and Teh, 2003; Hughes et al., 2004; Wu and Hua, 2008). These studies uncovered a wide variety of proteins that may or may not interact directly with menin. These proteins (menin interacting proteins or MIPs) can be grouped into four major functional classes: Class I, which includes transcription factors like JunD (Agarwal et al., 1999; Gobl et al., 1999; Heppner et al., 2001), NFκB (p50, p52 and p65) (Heppner et al., 2001), Smad3 (Kaji et al., 2001), BMP2 (bone morphogenic protein 2) (Sowa et al., 2004), IGFBP-2 (Insulin-like growth factor binding protein 2) (La et al., 2004), FANCD2 (fanconi anemia complementation group D2 protein) (Jin et al., 2003), Pem (a homeobox containing transcription factor) (Lemmens et al., 2001), cMyb (Jin et al.); Class II, which includes DNA damage and replication proteins such as RPA (replication protein A 1 and 2) (Sukhodolets et al., 2003); Class III, which includes cell cycle regulatory proteins such as CDK inhibitors (p18 and p27) (Milne et al., 2005), ASK (activator s-phase kinase)

(Schnepp et al., 2004), type III intermediate filaments (glial fibrillary acidic protein or GFAP and Vimentin) (Lopez-Egido et al., 2002); and Class IV, which includes transcriptional activators such as MLL1/2 (mixed lineage leukemia proteins) (Hughes et al., 2004; Yokoyama et al., 2004), RNA polymerase II phosphorylated carboxy terminal domain (Hughes et al., 2004), LEDGF (Yokoyama and Cleary, 2008), and CHD1 (chromo domain helicase I) (Chen et al., 2006b). Based on these studies it has been suggested that MIPs modulate the transcriptional activator/repressor functions of menin (Agarwal et al., 1999; Agarwal et al., 2003; Heppner et al., 2001; Kaji et al., 2001). While a direct role for the interaction of menin with all MIPs has yet to be validated *in vivo*, it is possible that MIPs regulate transcription by binding directly or indirectly to menin (Balogh et al., 2006; Chandrasekharappa and Teh, 2003; Wu and Hua, 2008). In addition, menin also binds to a putative tumor metastasis suppressor/nucleoside diphosphate kinase (Nm23), which stimulates the GTP hydrolyzing activity of menin (Ohkura et al., 2001). Moreover, menin also functions as a transcriptional co-activator of the nuclear receptor pathway by binding with estrogen receptor-alpha (ERalpha) in a hormone-dependent manner (Dreijerink et al., 2006). A non-specific DNA binding activity through the C-terminal NLS has also been reported for menin (La et al., 2004). Of the multitude of interactions reported for menin, it is the association with the SET1 family methyltransferases MLL1/2 that has generated a lot of interest due to their roles in hematopoiesis and leukemia.

4.5 Role of menin in Hematopoiesis

Menin is an essential component of MLL1/2 family complexes with specific roles in the maintenance of HOX gene expression patterns during hematopoiesis (Hughes et al., 2004; Yokoyama et al., 2004). Conditional knockouts of the MEN1 gene in mice decreases peripheral white blood cell counts as well as colony forming potential of bone marrow hematopoietic progenitors (Chen et al., 2006b). Recent work by Maillard et al., (2009a), suggests that while conditional menin knockouts have modest effects on hematopoiesis under steady-state conditions, more severe defects are observed in competitive transplantation assays and during drug-mediated chemoablation (Maillard et al., 2009). These studies suggest that menin functions as an essential regulator of hematopoietic stem cell (HSC) homeostasis specifically in situations of hematopoietic stress (Maillard and Hess, 2009). These phenotypes may be due, at least in part, to menin's role in regulating *HOX* gene expression. For example, small-interfering RNA (siRNA) mediated knockdown of menin or conditional MEN1 (-/-) knockout embryos show significant decreases in the expression levels of several HOX genes including HOXA9, HOXC6, and HOXC8 (Chen et al., 2006b; Hughes et al., 2004; Wu and Hua, 2008; Yokoyama et al., 2004). Interestingly, these hematopoietic defects are rescued by the ectopic expression of menin or its downstream targets HOXA9/MEIS1 (Chen et al., 2006b; Hughes et al., 2004). Evidence indicates that menin's interaction with MLL1/2 complexes is required for its role in transcription. For example, chromatin immunoprecipitation studies using antibodies specific to menin, MLL1, trimethylated H3K4 or CHD1 (which binds trimethylated H3K4) (Flanagan et al., 2005; Pray-Grant et al., 2005; Sims et al., 2005) have further established that menin co-localizes with these components at HOXA9 promoters and is required for transcriptional activation (Chen et al., 2006b; Wu and Hua, 2008). Furthermore, it has been shown that a subset of naturally occurring mutations in menin (H139D, A242V, and T344R) disrupts its association with MLL1 and fails to associate with MLL1-dependent H3K4 methyltransferase activity

(Hughes et al., 2004) (Refer to figure 3 for MEN1 mutations). These findings underscore the importance of the menin-MLL1 interaction in regulating HOX gene expression in hematopoiesis.

4.6 Role of menin in leukemogenesis

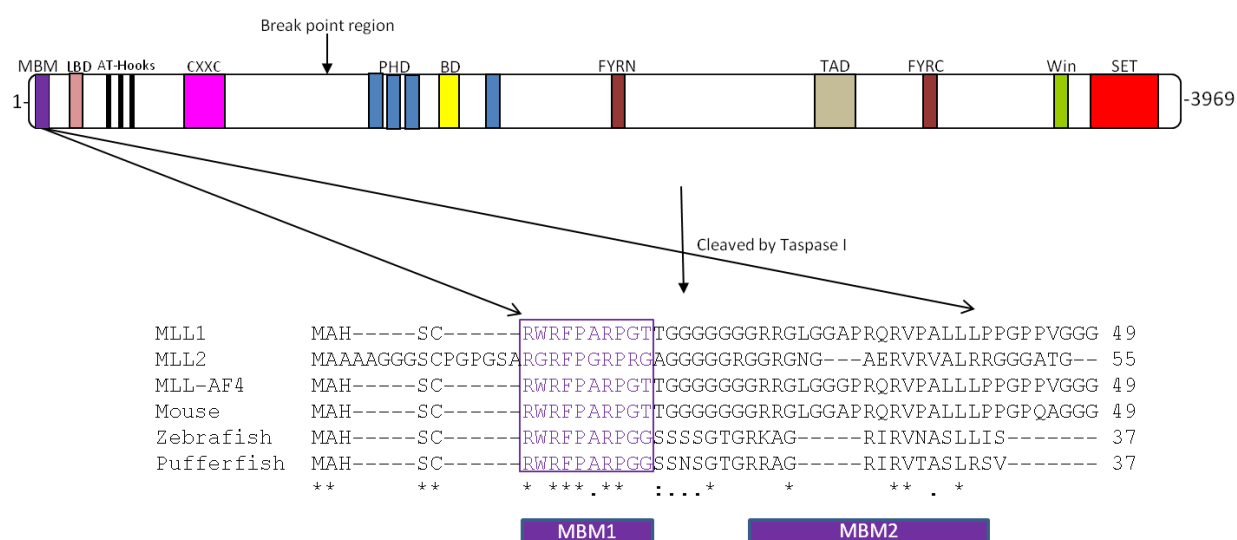
The first evidence for the involvement of menin in MLL1-associated leukemogenesis came from the initial biochemical studies carried out by Yokoyama et al., (2005) who identified a menin binding motif (MBM) located within the first 330 amino acids of MLL1, a region that is also retained in all types of MLL1 aberrations (Figures 1 and 2) (Yokoyama et al., 2005). Using leukemia cells that carry specific MLL1-translocations (MLL1-AF6, MLL1-ENL, MLL1-AF9, MLL1-AF10, and MLL1-GAS7) it was demonstrated that menin specifically associates with these MLL1-fusion proteins at the promoter of HOX genes such as: HOXA7, HOXA9 and HOXA10, which are constitutively expressed in several cases of acute leukemia (Chen et al., 2006b; Yokoyama et al., 2005; Yokoyama et al., 2004). Acute loss of menin reduces the aberrant HOX gene expression and abrogates the differentiation arrest associated with these MLL1-fusions (Yokoyama et al., 2005). Furthermore, conditional knockouts of the MEN1 gene suggests that menin is essential for the initiation and maintenance of MLL1-associated, but not other oncogene induced, myeloid transformations (Caslini et al., 2007; Chen et al., 2006b; Yokoyama et al., 2005). Together, these results demonstrate that MLL1-fusion proteins are dependent on menin for their oncogenic properties and raise the possibility that molecular therapies that target the menin-MLL1 interaction might be an effective strategy to treat leukemias.

While it is unclear how menin modulates the activities of wild-type and MLL1-fusion proteins, recent studies suggest that menin functions to recruit other proteins that are required for targeting MLL1 to downstream genes. For example, a recent study by Yokoyama and Cleary (2008) suggests that menin promotes LEDGF binding to MLL1. LEDGF contains a highly conserved PWWP motif that is required for MLL1's association with downstream target genes (Yokoyama and Cleary, 2008). In addition, menin amino acid substitutions that disrupt its interaction with LEDGF without affecting menin's interaction with MLL1 also display decreased Hoxa9 gene expression (Roudaia and Speck, 2008; Yokoyama and Cleary, 2008). These data indicate that part of menin's function is to stabilize the interaction between MLL1 and LEDGF. More recently, it has been shown that menin recruits the transcription factor cMyb to the MLL1 complex, which is required for recruitment of MLL1 to the Hoxa9 promoter (Jin et al., 2010). It has been shown that depletion of cMyb decreases the transforming potential of the MLL1-ENL fusion protein (Jin et al., 2010), suggesting that molecules that inhibit the menin-cMyb interaction may also be useful therapeutic agents.

4.7 Menin interacts with the N-terminus of MLL1 through an evolutionarily conserved Menin Binding Motif (MBM)

The interaction of menin with N-terminal sequences of wild-type MLL1 and MLL1-chimeric fusion proteins have been demonstrated by three independent reports (Caslini et al., 2007; Grembecka et al., 2010; Yokoyama et al., 2005). While the conclusions of these studies differ with respect to the exact length of the MLL1 fragment predicted to be involved in the interaction with menin, they have collectively identified a highly conserved "menin binding motif" (MBM) localized within the first 46 residues of MLL1. For example, Yokoyama et al.,

(2005) demonstrated that a consensus sequence (RXRFP), called the high-affinity MBM, is present between MLL1 amino acids 6-10. Sequence analysis reveals that the MBM is highly conserved among MLL1 orthologs and in MLL2 (residues 17-21) (Figure 3). Deletions of the MBM in the MLL1-ENL and MLL1-GAS7 fusion proteins abrogates the interaction with menin in 293T cells (Yokoyama et al., 2005). Furthermore, MLL1-ENL fusions that lack the MBM fails to induce acute myeloid leukemia in syngeneic recipient mice (Yokoyama et al., 2005). This failure is associated with impaired expression of HOXA7 and HOXA9 genes in the MBM-deleted MLL1-ENL transduced murine myeloid progenitors (Yokoyama et al., 2005). Moreover, MBM deletion mutants of MLL1-ENL lose their clonogenic potential and induce differentiation in leukemia blasts, a phenotype similar to the conditional knock out of menin or MLL1-ENL itself (Ayton and Cleary, 2003; Yokoyama et al., 2005; Zeisig et al., 2004). These studies demonstrate the importance of menin-MLL1 interaction in the pathogenesis of MLL1-associated leukemias.



The different functional domains in MLL1 are indicated and color coded as in Figure 1. The menin binding motif encompassing residues 5-44 (purple) along with LEDGF binding domain (LBD)(light red), AT-hooks (black), and the CXXC motif (pink) are retained by both MLL1-translocations and PTD mutations. The blow up region shows a ClustalW multiple sequence alignment of the high affinity menin binding motifs, MBM1 and MBM2, present in human (Q03164), mouse (NP_001074518), zebrafish (ACN88688), and pufferfish (AAC41377) MLL1s; and human MLL2 (O14686), and MLL1-AF4 (AAC37520) fusions (indicated in purple). MBM1 and 2 were identified based on three independent studies (Yokoyama et al., 2005; Caslini et al., 2007; Grembecka et al., 2010).

Fig. 4. Menin Binding Motifs (MBMs) are present in the wild-type MLL1, MLL1 chimeric fusions and partial tandem duplications

In an attempt to further characterize the physiological significance of the MLL1-menin interaction in acute leukemias, Caslini et al., (Caslini et al., 2007) demonstrated that MLL1 residues 5-44 are required for high affinity binding with menin. This MBM region spans the RWRFP motif (residues 6-10) and also includes a second region between MLL1 residues 35 and 44 that is necessary, but not sufficient, for high-affinity interaction with menin (Caslini et al., 2007). Internal deletions in the MLL1-AF9 fusion protein that lacked amino acids 5-15 (high affinity MBM) or 35-44 (low affinity MBM) failed to co-immunoprecipitate with menin from 293T cells (Caslini et al., 2007). Furthermore, Caslini et al., (2007) also demonstrated

that MLL1-AF9 MBM sequences were essential for the transformation of hematopoietic progenitors by the MLL1-AF9 fusion protein. Interestingly, the MLL1 constructs MLL1(2-167), MLL1(2-62), and MLL1(2-44) function as dominant negative inhibitors of the MLL1-menin interaction by titrating menin from the endogenous MLL1-AF9 protein resulting in reduced HOXA9 and MEIS1 expression and inhibition of the growth of transformed bone marrow progenitors (Caslini et al., 2007). Together, these results suggest that expression of dominant negative MLL1 constructs or peptide inhibitors that mimic the MLL1-menin interaction can inhibit the transforming potential of MLL1-fusion proteins by specifically down regulating the expression of target HOX genes. Unexpectedly, dominant negative constructs of MLL1 also inhibited the colony-forming ability of wild-type hematopoietic progenitors since these constructs also mimic the interaction surface between wild-type MLL1 and menin (Caslini et al., 2007). Collectively, these findings suggest that small molecule inhibitors that target menin-MLL1 interaction have a therapeutic potential to treat MLL1-associated leukemias, but with the caveat that normal hematopoiesis might also be impaired (Caslini et al., 2007).

4.8 MBM based peptides as novel therapeutic agents for acute leukemias with MLL1-rearrangements

Structural and biochemical studies that characterize the menin-MLL1 interaction in detail is an important step in the development of MBM-based small molecule inhibitors that can specifically help treat MLL1-mediated cancers. Grembecka et al., (2010) carried out a detailed biophysical characterization of the interaction between menin and MLL1 using a combination of NMR, Isothermal Titration Calorimetry (ITC) and Fluorescence Anisotropy (FP). They found that MLL1 binds menin with high affinity ($K_d \approx 10$ nM) utilizing two menin binding motifs (MBM1 and 2) located within the first 43 amino acids of MLL1 as previously suggested (Caslini et al., 2007; Yokoyama et al., 2005). Furthermore, peptides derived from the MBM1 (amino acids 5-14) and MBM2 (23-40) in MLL1 bind menin with interaction affinities of 53 nM and 1400 nM, respectively (Grembecka et al., 2010). Using a series of peptide competition experiments, MBM1 and MBM2 peptides were shown to displace a construct of MLL1 (amino acids 2-43) from bound menin in vitro with IC_{50} values of 0.5 μ M and 37 μ M, respectively (Grembecka et al., 2010). Moreover, based on transfer-nuclear overhauser effects (Tr-NOEs) based NMR experiments, it was further suggested that MBM1 interacts with menin in an extended conformation and that the binding is facilitated by hydrophobic residues Phe9, Pro10 and Pro13 (Grembecka et al., 2010). Substitution to alanine of these amino acid residues significantly impaired the binding of MLL1 constructs to menin (Grembecka et al., 2010). Together, these studies have identified MBM1 (which encompasses the consensus RWRFP) as a potential drug target for leukemias with MLL1 translocations.

5. Molecular targeting of MLL1-rearranged leukemias - Peptide inhibitors that target the activity of MLL1-AF4 and MLL1-AF9 fusion proteins

5.1 Clinical significance of t(4;11) and t(9;11) translocations

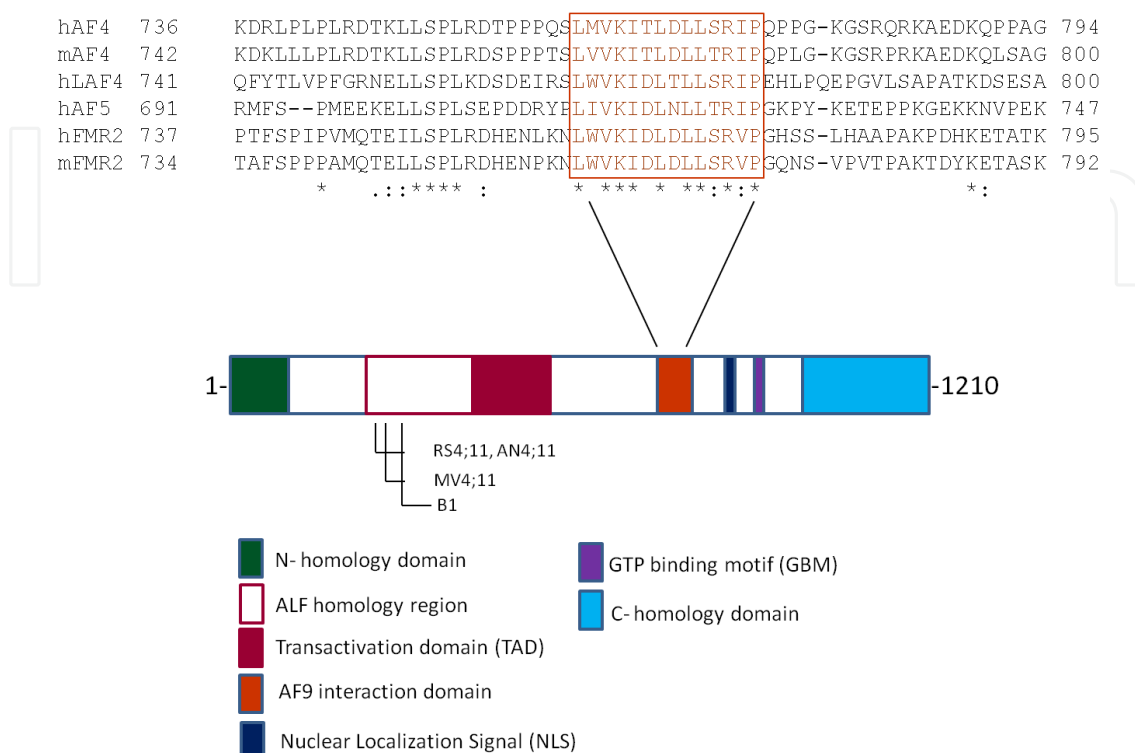
MLL1 translocations do not share a common structural motif or biochemical function. However, based on sequence similarities, the most commonly occurring MLL1 fusions can be grouped into three major gene families: AF10/AF17, ENL/AF9, and the largest family,

AF4/LAF4/AF5q31/FMR2 (Nilson et al., 1997). The most common translocation is t(4;11)(q21;q23) and is associated with more than 50% of acute leukemia cases in infants, and for 3-6% of cases in older children (Behm et al., 1996; Faderl et al., 1998; Heerema et al., 1999). The t(4;11) translocation results in leukemic blasts expressing phenotypic markers for ALL in 95% of the cases (Chen et al., 1993; Pui et al., 2003). t4:11 translocations result in cancers that often spread beyond the hematopoietic lineage and have a poor prognosis (Armstrong et al., 2002; Chen et al., 1993; Rubnitz et al., 1994a). The t(4;11) translocation retains the 5' portion of MLL1 gene containing the menin binding motif (MBM), AT hooks and the CXXC DNA binding motifs, which are fused in-frame to the 3' portion of the gene at the 4q21 locus called AF4 (Gu et al., 1992). The high occurrence rates of t(4;11) translocation in infants along with the poor prognosis and absence of chemotherapeutics to treat these leukemias highlight an urgent need for the development of inhibitors that specifically target the gain-of-function phenotypes associated with the MLL1-AF4 fusion. In this regard, inhibitors that target the interaction between AF4 and its partner protein AF9 have been developed recently and show promising results in inhibiting the transforming potential of leukemia cell lines bearing MLL1-AF4 or MLL1-AF9 translocations (Bennett et al., 2009; Palermo et al., 2008; Srinivasan et al., 2004). The biochemical studies that form the basis for these conclusions are summarized in this section.

5.2 Domain architecture and the functional roles of AF4 family

AF4, also known as AFF1/FEL, is a serine/proline-rich nuclear protein with crucial roles in B and T lymphocyte development. AF4 has several putative functional domains including the ALF (AF4/LAF4/FMR2 homology) domain, which mediates the interaction with a family of ubiquitin ligases called SIAH (seven in absentia homolog), a serine/proline-rich transcriptional activation domain (TAD), nuclear localization signals (NLS), a guanosine triphosphate (GTP) binding motif (GBM), and a C-terminal homology domain involved in intra-nuclear localization and binding to pre-mRNA splicing factors (Bensaid et al., 2009; Chen et al., 1993; Isnard et al., 2000; Melko et al.; Morrissey et al., 1993; Oliver et al., 2004) (Figure 5). AF4 is located at a fragile break-point region on chromosome 4 and is associated with a wide variety of chromosomal translocations. AF4 is a member of AF4/LAF4/AF5q31/FMR2 family of nuclear transcription factors (Gu et al., 1996; von Bergh et al., 2001; von Bergh et al., 2002) and also shows significant homology to the *Drosophila melanogaster* pair-rule gene *Lilliputian* (Su et al., 2001). Surprisingly, three of these family members (AF4/LAF4/AF5q31) are associated with infant leukemias involving reciprocal translocations with the MLL1 gene (Domer et al., 1993; Ma and Staudt, 1996; Taki et al., 1999). The second family member LAF4, isolated from Burkitt's lymphoma, is a lymphoid-specific transcription factor and has transcriptional activation domains and nuclear localization signals that are highly similar to AF4 (Ma and Staudt, 1996). Indeed, the MLL1-LAF4 fusion proteins also retain the TAD in LAF4, which can functionally substitute for the activation domain in MLL1, thereby contributing to the leukemogenic potential of these chimeric fusion proteins (Ma and Staudt, 1996). The third AF4 homologue AF5q31 was originally identified from infant acute leukemias with a (5;11)(q31;q13q23) translocation (Taki et al., 1999). Strikingly, each of these MLL1-AF4 gene family fusions manifest very similar clinical characteristics- that is, early onset, poor prognosis, and a mixed immunophenotype. However, the fourth AF4 family member, FMR2, is associated with mental retardation and is located in the folate-sensitive break-point region at chromosome X

band q28 (Gecz, Gedeon et al. 1996; Gu, Shen et al. 1996). FMR2 has not been reported to be a part of any MLL1 fusions.



Overview of the protein domain structure of human AF4 adapted from (Li et al., 1998; Srinivasan et al., 2004 and Melko et al., 2011). The different domains are highlighted: N-homology region with an unknown function (green), ALF (AF4/LAF4/FMR2 homology) region which includes the transcription activation domain (dark red), AF9 interaction region (orange), nuclear localization signals (dark blue), a putative consensus guanosine triphosphate (GTP) binding motif (purple), and C-terminal homology domain (light blue). The domains are not drawn to scale. The ClustalW multiple protein sequence alignment of the AF9 binding regions of AF4 family members: human AF4 (hAF4, Pubmed ID: P51825), mouse AF4 (mAF4, Pubmed: AAU93698), human LAF4 (hLAF4, PubMed ID: NP002276), human AF5 (hAF5, PubMed ID: Q9UHB7), human FMR2 (hFMR2, PubMed ID: BAC81113) and mouse FMR2 (mFMR2, PubMed ID: CAA04821) are shown with the AF9 interaction region boxed. Chromosomal break points are also indicated for cell lines carrying t(4;11) translocations.

Fig. 5. Domain representation of human AF4 highlighting the AF9 interaction region.

Despite the growing amount of clinical evidence suggesting the involvement of MLL1-AF4 family translocations in human acute leukemias, the molecular mechanisms by which the MLL1-AF4 fusion proteins induce neoplasia are not fully understood. One possibility is that the resulting MLL1-AF4 fusion protein has a gain-of function phenotype wherein the transcriptional activation domain of AF4 is fused to the DNA binding domains of MLL1 and alters the regulation of MLL1 dependent genes (Prasad et al., 1995). However, genetic studies using mouse knock-in models suggest that the two fusion proteins MLL1-AF4 and AF4-MLL1 resulting from the balanced reciprocal translocation between MLL1 and AF4 could independently contribute to the pathogenesis of t(4;11) mediated acute leukemias (Bursen et al.; Chen et al., 2006a; Isnard et al., 2000). Importantly, the AF4-MLL1 fusion protein retains the C-terminal portion of MLL1 including its SET domain, and is sufficient to induce leukemia in the absence of MLL1-AF4 (Bursen et al., 2010).

5.3 Molecular interaction network for AF4 fusion proteins

These studies raise the possibility that MLL1-AF4 and AF4-MLL1 participate in different sets of interaction networks as compared to the native full-length proteins (MLL1 and AF4) and result in gene expression signatures that are a representative of the physiological function of the fusion partners. This hypothesis is supported by the studies of Benedikt et al., (2011), who used affinity purified AF4 and AF4-MLL1 complexes from 293T cells to elucidate the subunit composition of the two complexes (Benedikt et al., 2011). Wild-type AF4 was purified in a complex containing the CDK9/Cyclin T heterodimer, which resembles the positive transcription elongation factor b (p-TEFb) (Benedikt et al., 2011; Estable et al., 2002) and also co-purifies with the wild type versions of two other known MLL1 fusion partners, AF9 and ENL, both of which bind to the C-terminus of AF4 family members (Benedikt et al., 2011; Erfurth et al., 2004; Mueller et al., 2009). Furthermore, ENL binding to AF4 creates a binding site for AF10, DOT1 methyltransferase and histone H3 (Benedikt et al., 2011; Mueller et al., 2007). Both the DOT1 mediated H3K79 methylation and the p-TEFb mediated phosphorylation of RNA Pol II C-terminal domain could lead to transcriptional elongation and is facilitated by the interaction of wild-type AF4 with these proteins (Benedikt et al., 2011; Bitoun et al., 2007). In contrast, the subunit composition of the AF4-MLL1 complex is different from the wild-type AF4 protein alone due to additional proteins that interact with the fused MLL1 C-terminal fragment. These proteins likely modulate the transcriptional activating properties of AF4-MLL1 fusion protein (Benedikt et al., 2011). MLL1-AF4 chimeric fusions on the other hand have an intact C-terminus of AF4 and therefore retain the transcriptional activation domains and its ability to interact with AF9 and ENL.

5.4 Functional significance of AF9/ENL family

Reciprocal translocations involving the AF9 (t(9;11)(p22;q23)) and ENL (t(11;19)(q23;p13)) genes are also associated with several cases of ALL and AML (Mitelman and Heim, 1992). Similar to AF4, AF9 also belongs to a family of serine/proline-rich transcription factors (Hemenway et al., 2001; Nakamura et al., 1993; Prasad et al., 1995) and shares significant similarity to ENL and the yeast protein ANC1 (Rubnitz et al., 1994b; Welch and Drubin, 1994). Interestingly, ANC1 has been demonstrated to be a part of the yeast RNA polymerase II complex, as well as the SWI/SNF nucleosome-remodeling complex, a macromolecular complex which functions as ATP-dependent chromatin remodeler (Cairns et al., 1996; Carlson and Laurent, 1994; Cote et al., 1994). The high degree of sequence similarity between human AF9/ENL and the yeast ANC1 protein has led to the hypothesis that AF9 and ENL may also interact with a human SWI/SNF remodeling complex similar to yeast ANC1, and the MLL1-AF9/ENL fusion proteins may retain these features. The biological functions of AF4, AF9 and ENL are not clearly understood, however, gene deletion studies in mice have demonstrated important roles for these proteins during development (Collins et al., 2002; Doty et al., 2002; Isnard et al., 2000). Furthermore, the endogenous ENL protein was purified as a part of a macromolecular complex (ENL associated protein complex or EAP) that also contains p-TEFb, DOT1 and AF4 and plays a putative role in transcriptional elongation (Bitoun et al., 2007; Mueller et al., 2007). Despite the fact that MLL1-AF4 translocations and MLL1-AF9/ENL fusions account for more than 50% of MLL1 11q23 associated leukemias (Burmeister et al., 2009; Meyer et al., 2009; Meyer et al., 2006), there exists no functional similarity between these MLL1 fusion partners. It is tempting to

hypothesize that MLL1 fusion proteins might hijack the p-TEFb/DOT1 mediated transcriptional elongation activity or the SWI/SNF dependent nucleosome remodeling activity through the fusion partner and result in constitutive target gene expression leading to leukemia. These studies suggest that pTEFb, DOT1 and the MLL1 fusion partners AF4/AF9/ENL are all molecular targets in the development of therapeutics that target MLL1-fusion mediated leukemias.

5.5 Domain mapping of the interaction region between AF4 and AF9

Co-localization studies carried out by (Erfurth et al., 2004) have established that the two most common MLL1 fusion partners AF4 and AF9 form a stable complex within the nucleus and are restricted to discrete nuclear foci called "AF4 bodies". AF4 bodies are nuclear speckle-like in appearance and are distinct from the nucleolus, cajal bodies, PML body or regions associated with DNA replication and repair (Erfurth et al., 2004). Using yeast two-hybrid screens it was demonstrated that the minimum motif in human AF4 required for binding AF9 encompasses 14 residues (761-774) that are proximal to the bipartite nuclear localization signal (NLS) in AF4 (refer to figure 5 for domain representation). Deletion of these residues completely abolishes the interaction between AF4 and AF9 and also results in diffuse AF4 bodies (Erfurth et al., 2004; Srinivasan et al., 2004). Site-directed mutagenesis studies reveals that bulky hydrophobic residues within the conserved AF9 binding region in mouse AF4 (mAF4) dictate its binding affinity to AF9 (Srinivasan et al., 2004). Using fluorescent tagged AF4 and AF9 constructs it was further demonstrated that both the 14 residue AF9 binding motif and the bipartite NLS was required for the punctate nuclear speckle distribution of AF4-AF9 complexes (Erfurth et al., 2004). Likewise, the terminal 93 residues in AF9 (and the terminal 84 in the case of ENL) were identified as the minimal region required for binding AF4 (Erfurth et al., 2004). Interestingly, the mutual interaction domains between AF4 and AF9 are highly conserved in the AF4 and AF9 homologues (refer to figure 5 for sequence alignment) (Erfurth et al., 2004; Srinivasan et al., 2004) and the AF4-AF9 interaction region is retained by the MLL1 fusion proteins (MLL1-AF4 and MLL1-AF9) (Dobson et al., 1999; Domer et al., 1993; Erfurth et al., 2004), suggesting that AF4-AF9 interaction might be an important step in the pathogenesis associated with these MLL1 fusions. The functional significance of the interaction between AF4 and AF9 is unclear; however, the co-localization of these two proteins to specific sub-nuclear foci suggests that AF4-AF9 interaction might be required for normal cellular functions as well as in the pathogenesis of MLL1-AF4 or MLL1-AF9 associated leukemias (Erfurth et al., 2004). Furthermore, the presence of this interaction region in MLL1-AF4 and MLL1-AF9 fusion proteins, and the ability of MLL1-AF4 fusions to alter the localization of endogenous AF9 also suggest that AF4-AF9 protein complex is a pharmacological target for leukemia therapy (Erfurth et al., 2004; Srinivasan et al., 2004).

5.6 A synthetic peptide PFWT disrupts the interaction between AF4 and AF9

Based on the initial mapping studies of the AF9 binding region in AF4, a synthetic peptide, designated "PFWT" that mimics the interaction region was developed and tested for its ability to disrupt the AF4-AF9 interaction both in vitro and in vivo (Erfurth et al., 2004; Srinivasan et al., 2004). The initial PFWT peptide developed in this study was based on the highly conserved AF4-AF9 interaction sequence in the mouse FMR2 protein and encompasses residues (759-771) (see Figure 5 for the sequence alignment of the AF9

interaction region in AF4 family members). To enable nuclear uptake, the PFWT peptide was conjugated to a penetratin transporter sequence at its N-terminus (Srinivasan et al., 2004). Using pull-down assays with GST-tagged AF4 and biotinylated AF9, it was shown that this PFWT peptide disrupts the interaction between human AF4 and AF9 in a concentration dependent manner (Srinivasan et al., 2004). However, a control peptide (containing amino acid substitutions at V763E and I765S) did not interfere with the binding of AF4-AF9 complex (Srinivasan et al., 2004). The specificity of PFWT peptide against AF4-AF9 complexes was further demonstrated by its inability to disrupt the interaction between AF9 and two other proteins, the Polycomb protein (MPC3) and the mouse homolog of BCL-6 co-repressor (mBCoR), both of which interact through the C-terminus of AF9 (Hemenway et al., 2001; Srinivasan et al., 2003; Srinivasan et al., 2004). Furthermore, the PFWT peptide was shown to be readily taken up by NIH3T3 cells and disrupts the co-localization of AF4-AF9 in vivo (Srinivasan et al., 2004). These results corroborated the in vitro findings that the PFWT peptide has the ability to specifically disrupt the interaction between AF4 and AF9 protein complexes.

5.7 PFWT peptide inhibits the cell proliferation of leukemia cell lines with t(4;11) and t(9;11) translocations

Based on these observations it was further predicted that the PFWT peptide will have the ability to inhibit the proliferation of leukemia cell lines that carry a t(4;11)(q21;q23) or t(9;11)(p22;q23) translocations (Bennett et al., 2009; Palermo et al., 2008; Srinivasan et al., 2004). As expected, PFWT peptide specifically inhibits the proliferation of leukemia cell lines B1, MV4-11 and RS4;11 (Cohen et al., 1991; Lange et al., 1987; Stong et al., 1985) that harbor the MLL1-AF4 translocation (Bennett et al., 2009; Palermo et al., 2008; Srinivasan et al., 2004). Interestingly, the PFWT peptide also inhibits the survival capacity of KP-L-RY cell lines (Cohen et al., 1991) that are characterized by a t(5;11) translocation associated with MLL1-AF5q31 fusions (Srinivasan et al., 2004). In contrast to cell lines that carry either the MLL1-AF4 or MLL1-AF5q31 translocations, the PFWT peptide shows mixed effects in the inhibition of the proliferative capacity of leukemia cell lines that carry MLL1-AF9 translocations (Palermo et al., 2008; Srinivasan et al., 2004). For instance, based on the study by (Srinivasan et al., 2004), the PFWT peptide failed to inhibit the growth of THP-1 cells (Tsuchiya et al., 1980) that are associated with MLL1-AF9 translocations. However, a more recent study demonstrated that Molm13 leukemia cells that are also associated with MLL1-AF9 translocations are sensitive to treatments with PFWT peptide in a dose dependent manner (Palermo et al., 2008). While these findings suggest that there are differences in the inhibitory properties of the PFWT peptide towards cell lines that carry a similar translocation, it highlights the fact that these leukemias might operate through additional pathways that lead to misregulated gene expression profiles (Palermo et al., 2008; Srinivasan et al., 2004). Furthermore, the specificity of PFWT peptide in inhibiting leukemia cells with MLL1-AF4 and MLL1-AF9 mutations is corroborated by the findings that the MOLT-4 (T-ALL) cell line (Minowada et al., 1972) that does not contain a MLL1-AF4 translocation is not affected by the PFWT peptide even at higher doses (Srinivasan et al., 2004). However, the Reh (B-precursor) cell lines (Koziner et al., 1985) that do not carry a MLL1-AF4 chimeric fusion are susceptible to the PFWT peptide (Srinivasan et al., 2004). These results suggest that some cell lines might require the function of normal AF4-AF9 complexes for its carcinogenesis and these native complexes are also targeted by the PFWT peptides

(Srinivasan et al., 2004). Nevertheless, these findings demonstrate the wide range of specificity for the PFWT peptide and suggest that PFWT-like peptides are promising lead compounds in the development of treatment regimens for the most frequent 11q23 translocations. The pharmacological importance of PFWT based peptides is further enhanced by the fact that these peptides have little effect on the number and colony forming potential of hematopoietic progenitor cells (Srinivasan et al., 2004), suggesting that PFWT-like peptides may have fewer side effects.

5.8 PFWT peptide as a lead compound in the treatment of MLL1-rearranged leukemias

While initial studies suggest that the growth inhibitory properties of the PFWT peptide is mediated through apoptosis (Srinivasan et al., 2004), recent work by (Palermo et al., 2008) demonstrates that PFWT peptide induces cell death by necrosis in MV4-11 and Molm13 cell lines. Necrotic cell death was further demonstrated by the changes in plasma membrane integrity in the absence of traditional apoptotic markers: nuclear disintegration, caspase activation, DNA fragmentation or mitochondrial membrane depolarization (Palermo et al., 2008). Given the resistance of t(4;11) translocations to conventional pro-apoptotic chemotherapeutic drugs, PFWT mediated necrotic cell death is an attractive alternative strategy to treat these acute leukemias. More recently, Bennett et al., (Bennett et al., 2009) demonstrated that the PFWT peptide in combination with standard chemotherapeutic agents such as etoposide, 17AAG (17-(allylamino)-17-demthoxygeldamycin), cytarabine, and Flt-3 kinase inhibitor results in synergistic cytotoxicity in MV4-11 cells that harbor t(4;11) translocations. Furthermore, cell death was mediated through both apoptotic and necrotic pathways suggesting that PFWT peptides could be useful in combinatorial therapy to treat leukemia cell lines that are relatively resistant to current apoptotic drugs (Bennett et al., 2009; Campo Dell'Orto et al., 2007; Nakanishi et al., 2007; Wiederschain et al., 2005). Together, these findings emphasize that PFWT peptides inhibit the proliferation of t(4;11), t(5;11) and t(9;11) leukemia cell lines by specifically disrupting the association of AF4 or AF5q31 with AF9 and serves as a starting point for the development of more effective therapeutic strategies. In the absence of a crystal structure that clearly demonstrates the interaction mode between AF4 and AF9 proteins, systematic site-directed mutagenesis experiments can help identify amino acid positions that are more important for the overall binding affinity. Such an approach has the potential to help identify additional PFWT-based peptido-mimetic compounds that have better inhibitory properties than the initial PFWT peptide and prevent the potential toxic side effects that could occur at high doses of the PFWT peptide.

6. Win motif-based inhibitors that target the assembly and enzymatic activity of the MLL1 core complex

6.1 Novel strategies that target the histone methyltransferase activity of MLL1 amplifications and MLL1-PTDs

Growing evidence suggests that increased HOXA gene expression associated with MLL1 amplifications and MLL1-PTDs underlie the pathogenesis of these leukemias (Basecke et al., 2006; Dorrance et al., 2006). HOXA gene expression is dependent on the histone methyltransferase activity of MLL1 (Milne et al., 2002), and given the increased H3K4 methylation observed in MLL1-PTDs (Dorrance et al., 2008; Dorrance et al., 2006), inhibitors

that down-regulate the histone methyltransferase activity of MLL1-PTD have the potential to reverse this aberrant epigenetic program. Until recently, inhibitors that specifically target MLL1's H3K4 methyltransferase activity have been difficult to develop due to lack of a clear understanding of MLL1's structure and function. However, recent findings have established that H3K4 methylation levels are precisely regulated by the function of two independent methyltransferases: the MLL1 SET domain and a novel multi-subunit enzyme, WDR5-RbBP5-Ash2L-DPY30 (WRAD), that lacks sequence homology to known methyltransferases (Patel et al., 2009; Patel et al., 2011). Because multiple methylation on H3K4 is catalyzed by two different enzymes, it is interesting to speculate that inhibitors that prevent the association of two methyltransferases will have clinical significance in the treatment of acute leukemias that are characterized by aberrant H3K4 methylation (Dorrance et al., 2008; Dorrance et al., 2006).

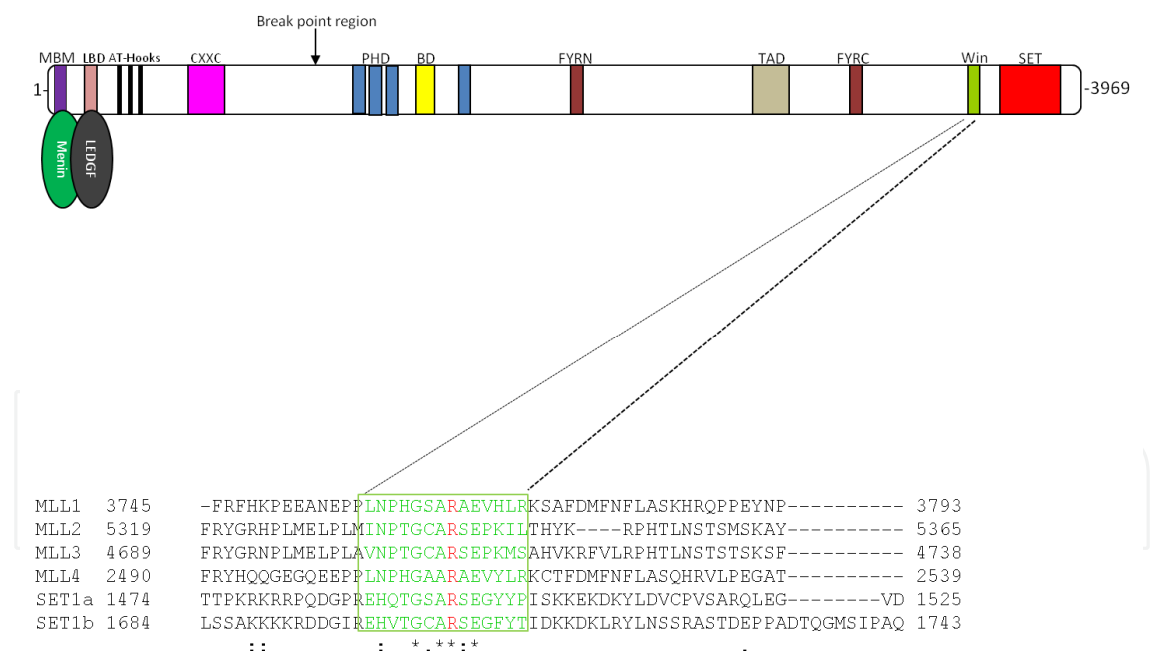
6.2 WDR5 is central for the assembly and H3K4 methylation activity of MLL1 core complex

The WD-40 repeat protein WDR5 is a conserved component of MLL1 family complexes ranging from yeast to humans and has been shown to be crucial for the assembly and H3K4 dimethylation activity of MLL1 core complex HOX (Dou et al., 2006; Patel et al., 2008a; Patel et al., 2008b). Consistent with the role of WDR5 in the regulation of degree of H3K4 methylation, siRNA mediated knock-down of WDR5 in mammalian cells results in a global decrease in the levels of H3K4 di- and trimethylation, down-regulation of HOXA9 and HOXC8 genes, and defects in hematopoiesis and development (Dou et al., 2006; Wysocka et al., 2005). WDR5 knock down and MLL1 Δ SET mice show similar phenotypes suggesting that MLL1 and WDR5 function together to regulate H3K4 di/trimethylation and HOX gene expression in vivo (Terranova et al., 2006; Wysocka et al., 2005). Furthermore, growing evidence suggest that WDR5 interacts directly with MLL1 or other SET1 family members and functions as a scaffold to bridge the interactions between MLL1 and rest of the components of MLL1 core complex (Dou et al., 2006; Patel et al., 2008b; Trievel and Shilatifard, 2009; Wysocka et al., 2005). Indeed, MLL1 and WRAD enzymatic complexes fail to associate in the absence of WDR5 (Dou et al., 2006; Patel et al., 2008b), suggesting a crucial role for WDR5 in the assembly and H3K4 methylation activity of MLL1 core complex.

6.3 WDR5 recognizes a conserved arginine containing sequence in the N-SET region of MLL1

Previous studies have suggested that WDR5 functions within the MLL1 core complex as a histone "effector" or "presenter" domain, a domain that specifically recognizes methylated histones (Couture et al., 2006; Han et al., 2006; Ruthenburg et al., 2006; Schuetz et al., 2006; Trievel and Shilatifard, 2009; Wysocka et al., 2005). However, recent studies by Patel et al., (2008a and b) and Song and Kingston., (2008) demonstrate that WDR5 recognizes a conserved arginine containing motif in the N-SET region of MLL1 called the WDR5 interaction (*Win*) motif (Patel et al., 2008b), thereby promoting the assembly and the H3K4 dimethylation activity of the MLL1 core complex (Patel et al., 2008a; Patel et al., 2008b; Song and Kingston, 2008) (Figure 6). Based on sedimentation velocity analytical ultracentrifugation experiments, Patel et al., (2008b) demonstrated that an MLL1 construct (encompassing residues 3745-3969) containing the highly conserved *Win* motif binds to WDR5 as a 1:1 complex with a dissociation constant of 120nM. Whereas, a shorter construct in MLL1 (encompassing residues 3811-3969) that lacks the *Win* motif sequence fails to associate with WDR5 (Patel et al., 2008b). In their

efforts to further map the interaction region between MLL1 and WDR5, the two groups showed that the conserved *Win* motif closely resembles the sequence surrounding arginine 2 of histone H3 and is highly conserved among metazoan MLL1 orthologs and other SET1 family members (Patel et al., 2008b; Song and Kingston, 2008) (Figure 6). Using a combination of analytical ultracentrifugation and MALDI-TOF mass spectrometry, it was demonstrated that the conserved arginine (R3765) of the MLL1 *Win* motif is crucial for the interaction with WDR5 and that substitution of R3765 with alanine in MLL1 abolishes the interaction between MLL1 and the WRAD sub-complex, which also results in the loss of the H3K4 dimethylation activity of the MLL1 core complex (Patel et al., 2008b). However, replacement of other residues such as serine 3763 and glutamate 3767 in the conserved *Win* motif sequence only modestly weakens MLL1's interaction with the WRAD sub-complex (Patel et al., 2008b). Furthermore, the MLL1 R3765A mutant fails to co-immunoprecipitate the rest of the core complex components RbBP5 and Ash2L from HeLa cells, consistent with a central role for the MLL1 *Win* motif in the assembly of MLL1 core complex in vivo (unpublished results from Lee and Skalnik). These results have led to a model in which the conserved *Win* motif of MLL1 and other metazoan SET1 family members functions to bind the WDR5 component of the WDR5-RbBP5-Ash2L sub-complex, which is required for the assembly and H3K4 dimethylation activity of the MLL1 core complex (Cosgrove and Patel, 2010; Trievel and Shilatifard, 2009). These observations suggest that targeting MLL1-WDR5 interaction might be an effective strategy to down-regulate H3K4 dimethylation and HOX gene expression mediated by the MLL1 core complex.



Schematic representation of the *Win* motif present in SET1 family members. At the top the domain architecture of wild-type MLL1 is shown and color coded as in Figure 1. The six-residue *Win* motif is present within the N-SET region of MLL1 (amino acid residues 3762-3767). The lower inset (green) shows the ClustalW multiple sequence alignment of the *Win* motif present in human SET1 family members: MLL1 (Q03164), MLL2 (O14686), MLL3 (AAK00583), MLL4 (Q9UMN6), SET1a (O15047), and SET1b (Q9UPS6). The conserved arginine of the *Win* motif is highlighted in red.

Fig. 6. WDR5 Interaction (*Win*) motif is highly conserved among human SET1 family members

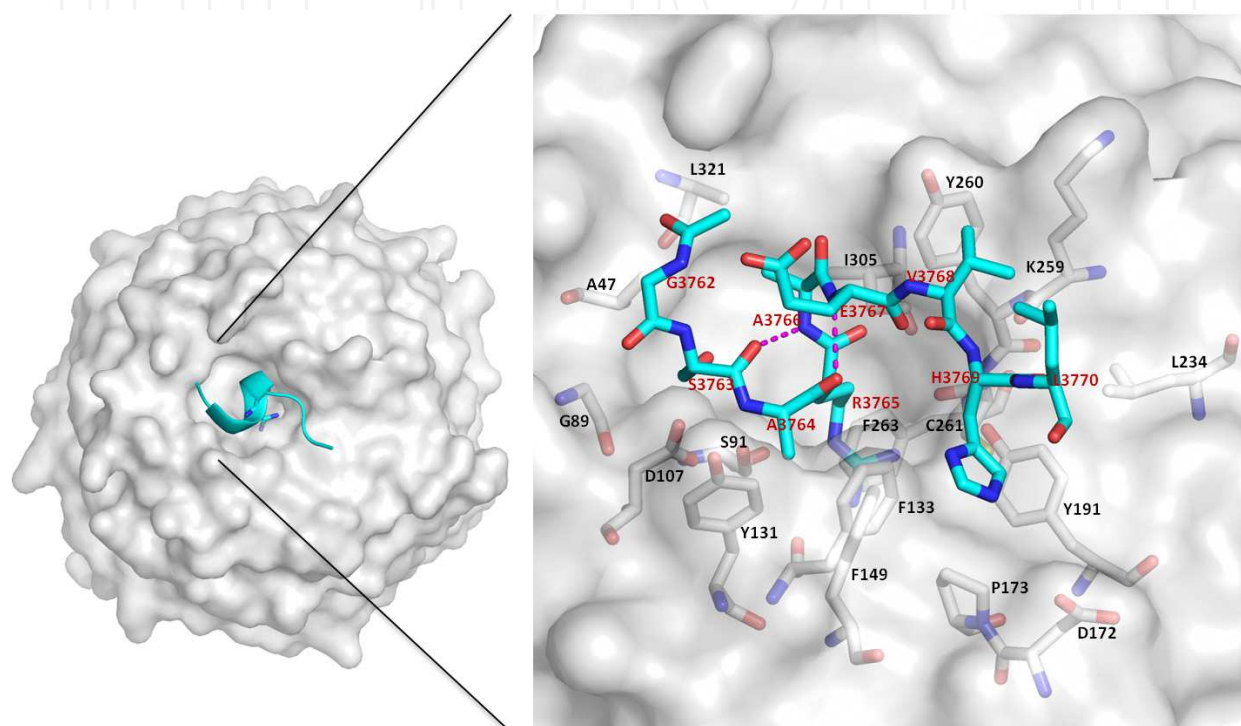
6.4 Peptides derived from the MLL1 *Win* motif specifically inhibit the H3K4 dimethylation activity of MLL1 core complex

Based on mapping studies of the WDR5 binding region in MLL1, Patel et al., (2008b) developed a peptide, designated the MLL1 *Win* motif peptide (3762-3773) that can bind WDR5 with high affinity ($K_d=1700\text{nM}$) and disrupt the assembly of the MLL1 core complex in vitro (Patel et al., 2008a; Patel et al., 2008b). Consequently, this 12-residue MLL1 *Win* motif peptide inhibits the in vitro H3K4 dimethylation activity of the MLL1 core complex in a dose dependent manner by competing with wild-type MLL1 for the arginine binding pocket in WDR5 (Patel et al., 2008b). However, a control p53 peptide that has an arginine in a different sequence context failed to inhibit the H3K4 dimethylation activity even with a 60-fold excess, suggesting that *Win* motif peptides are highly specific inhibitors of MLL1 family complexes (Patel et al., 2008b). Furthermore, recent unpublished results from our lab demonstrate that peptides derived from other human SET1 family members bind WDR5 with dissociation constants ranging from 50nM-1700nM (in preparation). In accordance with the binding studies, MALDI-TOF mass spectrometry based methylation assays further demonstrate that other human SET1 family *Win* motif peptides are significantly more potent inhibitors of the H3K4 dimethylation activity of the MLL1 core complex as compared to the MLL1 *Win* motif peptide (Dharmarajan and Cosgrove, unpublished results). These studies suggest that the MLL1 *Win* motif based peptide represents an excellent starting point for the design of lead compounds that would specifically disrupt the interaction between MLL1 and WDR5 and inhibit the H3K4 dimethylation activity of MLL1 core complex in vivo.

6.5 MLL1 *Win* motif peptide binds to WDR5 by adopting a 3_{10} -helical conformation

Design of *Win* motif based inhibitors with better efficiency could be greatly enhanced by determining the protein structural features within the *Win* motif that are required for the high affinity binding to WDR5. To facilitate the process of structure-based drug design and to understand the molecular basis for MLL1-WDR5 interaction, two independent groups determined the three-dimensional structure of WDR5 bound to a peptide derived from the MLL1 *Win* motif (Patel et al., 2008a; Song and Kingston, 2008). The structures reveal that MLL1 *Win* motif peptide binds WDR5 by adopting a partial 3_{10} -helical conformation (Figure 7) (Patel et al., 2008a; Song and Kingston, 2008). The conserved *Win* motif residues (3762-3767) all participate in the formation of 3_{10} -helix with two-intramolecular $i \rightarrow i+3$ main-chain hydrogen bonds stabilizing this conformation (Figure 7). MLL1 *Win* peptide binding is further stabilized by the insertion of conserved R3765 of MLL1 into a central water filled tunnel of WDR5 (Patel et al., 2008a; Song and Kingston, 2008). The structures also demonstrate that the side chain guanidinium of R3765 is sandwiched between two aromatic rings from WDR5- residues Phe-133 and Phe-263. R3765 is also stabilized by an extensive network of hydrogen bond, pi-pi and cation-pi, and hydrophobic interactions (Patel et al., 2008a; Song and Kingston, 2008) (Figure 7). These structural studies further corroborate the role of R3765 in mediating the interaction with WDR5 and explain the high sequence conservation of this arginine within SET1 family *Win* motifs. Accordingly, structures of WDR5 bound to *Win* motif peptides derived from other human SET1 family members reveal that WDR5 recognizes the different SET1 family *Win* motifs using the same arginine binding pocket as previously described for MLL1 (Dharmarajan and Cosgrove, unpublished results). However, the different *Win* motif peptides also participate in additional sets of interactions that might contribute to their differential inhibitory properties and suggest a framework for

the use of *Win* motif peptides as lead compounds for drug development (Dharmarajan and Cosgrove, unpublished results). While the *Win* peptide binding mode is similar to the previously determined structures of WDR5 bound to histone peptides, which bind by inserting R2 of histone H3 into the central tunnel in WDR5 (Couture et al., 2006; Han et al., 2006; Schuetz et al., 2006), the SET1 family *Win* motif peptides participate in more favorable interactions with WDR5 (Patel et al., 2008a; Song and Kingston, 2008). These findings suggest that peptidomimetics based on the *Win* motif sequence can specifically bind WDR5 and down-regulate the activity of MLL1 core complex.



Three-dimensional structure of WDR5 (PDB code 3EG6) in complex with MLL1 *Win* motif peptide (3762-3773). a) On the left, the MLL1 *Win* motif peptide (cyan) is shown bound to the central tunnel in WDR5 (white). MLL1 *Win* motif peptide binds WDR5 by adopting a 3_{10} -helical conformation. On the right, blow up of the peptide binding site with MLL1 residues indicated in red and WDR5 residues indicated in black. Atom coloring: oxygen (red), nitrogen (blue), carbon (cyan for *Win* motif residues and grey for WDR5 residues).

Fig. 7. Crystal structure of MLL1 *Win* motif peptide bound to WDR5

6.6 Analysis of the binding of MLL1 *Win* motif and histone H3 peptides to WDR5: identification of key structural elements required for binding WDR5

To facilitate the design of small-molecule inhibitors of MLL1-WDR5 interaction, Karatas et al (2010) performed a systematic deletion analysis on the original 12-residue MLL1 *Win* motif peptide (3762-3773) and showed that a three residue sequence composed of Acetyl-ARA-NH_2 is the minimal motif that is required for its interaction with WDR5 (Karatas et al., 2010). The acetyl group on the N-terminus is essential as its removal results in the loss of binding.

The Acetyl-ARA-NH_2 peptide binds WDR5 in peptide competition experiments with similar affinity to that of the original 12 residue MLL1 *Win* motif peptide (~ 120 nM) (Karatas et al., 2010; Patel et al., 2008b). Moreover, systematic mutagenesis and molecular dynamics

simulations further suggest that Acetyl-ARA-NH_2 peptide could recapitulate the two intramolecular $i \rightarrow i+3$ main chain hydrogen bonds that are present in the original 12-residue MLL1 *Win* motif peptide. Absence of one or both of these hydrogen bonds significantly weakens the interaction affinity between the 3-residue peptide and WDR5 (Karatas et al., 2010). These results suggest that Acetyl-ARA-NH_2 peptide binds WDR5 by adopting a partial 3_{10} -helical conformation similar to that of the 12-residue MLL1 *Win* motif peptide and participates in similar sets of interactions (Karatas et al., 2010; Patel et al., 2008b). However, the most potent inhibitor derived from the MLL1 *Win* motif peptide sequence is the acetyl-10mer ($\text{acetyl-ARAEVHLRKS-NH}_2$) encompassing residues 3764-3773, which binds WDR5 with a $K_i = 3\text{nM}$ (Karatas et al., 2010). These results suggest that sequences outside of the ARA sequence contribute to the affinity and would likely increase specificity. In addition, replacement of alanine at the +1 position (1 residue C-terminal to R3765) with a threonine in the acetyl-10mer ($\text{acetyl-ARTEVHLRKS-NH}_2$) or 3-mer (Acetyl-ART-NH_2) results in a 3-to-6-fold increase in binding affinity to WDR5, respectively (Karatas et al., 2010). In summary, these experiments suggest that the Acetyl-ARA-NH_2 motif anchors the peptide to the arginine binding pocket of WDR5 and that the 3_{10} -helical conformation is crucial for interaction. In addition, sequences N- and C-terminal to the ARA motif are important for increasing binding specificity. It remains to be determined if the Acetyl-ARA-NH_2 peptide actually inhibits the WDR5-MLL1 interaction within the context of the assembled MLL1 core complex.

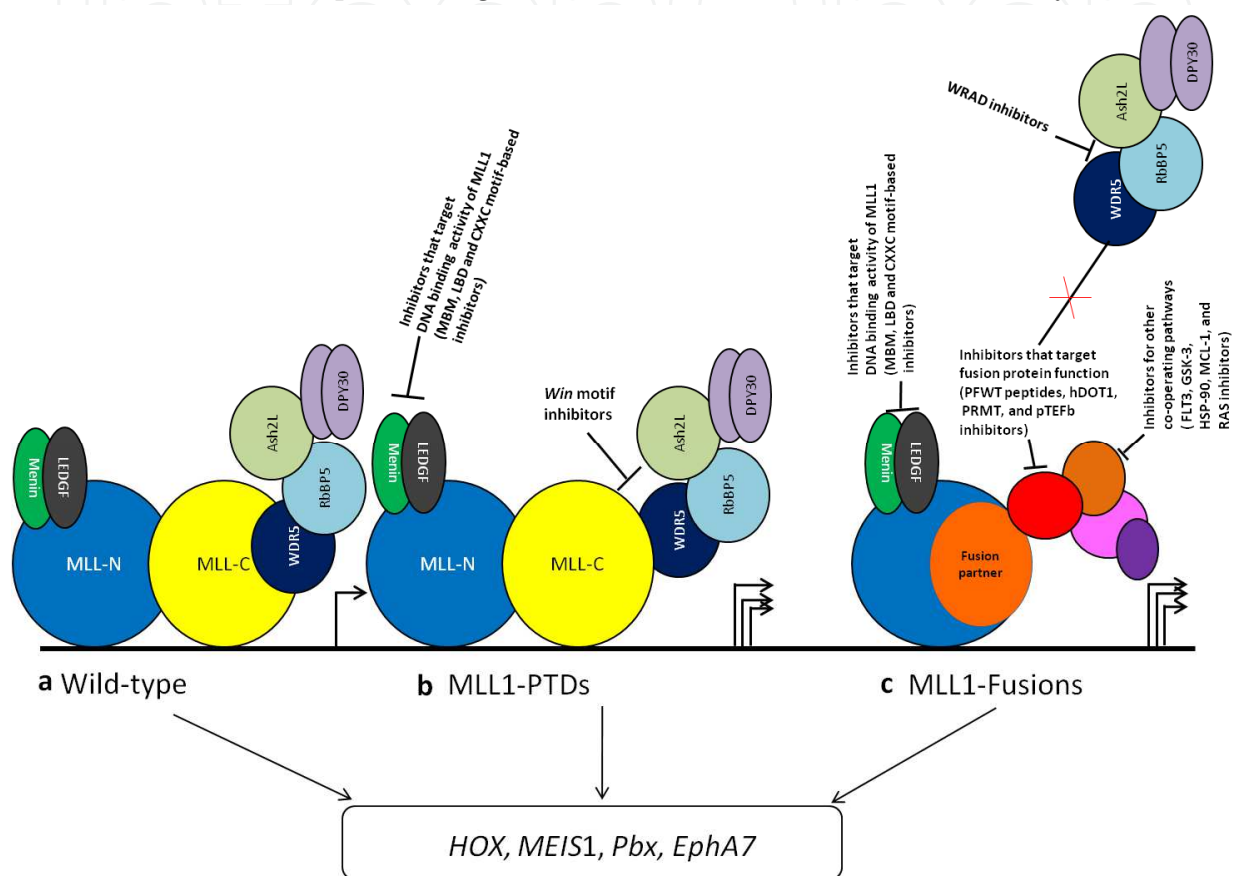
6.7 *Win* motif based inhibitors - a novel class of compounds with potential to treat Acute Myeloid Leukemia

The extensive biochemical characterization of the peptide derived from the MLL1 *Win* motif have demonstrated that these peptidomimetic compounds have the potential to inhibit the H3K4 methyltransferase activity of the MLL1 core complex by specifically disrupting the association of MLL1 with the WRAD sub-complex (Patel et al., 2008b). Furthermore, the crystal structures of WDR5 bound to human SET1 family *Win* motif peptides provides a rationale for the design of new peptides and non-peptide mimetics with better inhibitory properties. The structures also reveal that conserved residues from the MLL1 *Win* motif (GSARAE) form a cyclical shaped 3_{10} -helix that fits snugly into the outer opening in WDR5 and raises the possibility that other cyclic peptidomimetic compounds might also bind WDR5 with similar or better inhibitory properties. The efficacy of *Win* motif based inhibitors in down regulating the H3K4 methylation activity of MLL1 core complex *in vitro* has yet to be demonstrated *in vivo*. However, based on the *in vitro* studies it is expected that the *Win* motif based peptides will have the ability to down regulate the increased H3K4 dimethylation, and aberrant HOX gene expression associated with MLL1-PTDs and gene amplification mutations in MLL1. Hence, the MLL1 *Win* motif peptide is a novel "first in class" inhibitor that is expected to have the ability to specifically regulate H3K4 dimethylation levels in cells without perturbing H3K4 monomethylation, or the methylation activities of other H3K4 methyltransferases.

7. Conclusions and future perspectives - are there other molecular targets for leukemia therapy?

Over the past decade, we have witnessed remarkable strides towards understanding the fundamental mechanisms of MLL1-mediated transcription and leukemogenesis, and many

targets that are likely to be biomedically important are beginning to be characterized (Dou and Hess, 2008; Liedtke and Cleary, 2009; Marschalek, 2010). Genetic, biochemical and structural studies have demonstrated that several MLL1 partner proteins (AF4, AF9, AF10, ENL, and EEN) are either directly or indirectly associated with macromolecular complexes involved in transcriptional initiation and elongation (Bitoun et al., 2007; Cheung et al., 2007; Marschalek; Mueller et al., 2007; Mueller et al., 2009; Okada et al., 2005; Okada et al., 2006). Based on these studies, it was also suggested that leukemogenic properties of these MLL1-fusions are mediated in part through their association with the histone methyltransferases:



Cartoon representation of the different forms of MLL1 (a. wild-type, b. PTDs, and c. chromosomal translocations) and a summary of emerging potential molecular targeted therapies for mixed lineage leukemia. Key molecular targets include: i) inhibitors that disrupt the association of MLL1 with its target genes (MBM-based or CXXC motif-based inhibitors), ii) inhibitors that target the assembly of MLL1 core complex (*Win* motif-based inhibitors), iii) inhibitors that target the functions of fusion proteins (PFWT-like peptides, hDOT1L inhibitors, pTEFb kinase inhibitors), iv) WRAD sub-complex inhibitors and v) Inhibitors that target other co-operating pathways in leukemia (GSK-3, FLT3, MCL-1, and Ras). MLL-N (blue) and MLL-C (yellow) fragments are shown as localized to a hypothetical MLL1 target gene. In a). menin (green), LEDGF (dark grey) and WRAD sub-complex associate with wild-type MLL1 to regulate the normal expression (indicated by a single arrow) of MLL1 target genes. Target gene expression is dysregulated in MLL1-PTDs and MLL1-fusions (b and c) as indicated by multiple arrows. b) MLL1-PTDs retain all the functional interactions as seen in wild-type MLL1 and c) MLL1-fusions do not retain the MLL-C fragment and many interacting proteins.. Figure 8 was adapted from Liedtke and Cleary (2009).

Fig. 8. Potential protein:protein interaction targets for the development of novel therapies for MLL1-associated leukemias

hDOT1 (Bitoun et al., 2007; Okada et al., 2005; Okada et al., 2006) and protein arginine methyltransferase-1 or PRMT1 (Cheung et al., 2007). These studies have led to the proposal of the “MLL1 web hypothesis”, which states that MLL1 fusion partners are components of larger macromolecular complexes that are involved in transcriptional activation and/or elongation (Erfurth et al., 2004). Molecular therapies directed at the interactions of these proteins within the “MLL1 web hypothesis” or inhibiting the activity of associated histone modifying enzymes are emerging as promising targets (Dou and Hess, 2008; Liedtke and Cleary, 2009; Marschalek, 2010). In addition to targeting the function of MLL1-fusion proteins, inhibitors that target the association of MLL1-fusion proteins to target DNA might also have potential therapeutic implications. In this regard, inhibitors that disrupt the interaction between MLL1’s CXXC motif and unmethylated CpG containing target DNA are being investigated as molecular drug targets in MLL1-related leukemias (Allen et al., 2006; Cierpicki et al., 2010). In addition to these molecular targets, recent studies have also identified other cooperating pathways such as positive transcription elongation factor-b (pTEFb) recruitment (Marschalek, 2010; Mueller et al., 2007; Mueller et al., 2009; Shapiro, 2006), activation of FMS-like tyrosine kinase 3 (FLT3) (Armstrong et al., 2003; Brown et al., 2005; Stubbs et al., 2008; Yao et al., 2005), glycogen synthase kinase 3 (GSK3) (Wang et al., 2008), heat shock protein-90 (HSP-90) (Yao et al., 2005; Yocum et al., 2006), myeloid cell leukemia sequence-1 (MCL-1) expression (Chen et al., 2009), and RAS pathways (Liang et al., 2006) that are implicated in MLL1-induced leukemogenesis. The therapeutic significance of these molecular targets in MLL1-therapy were reviewed recently (Dou and Hess, 2008; Liedtke and Cleary, 2009; Marschalek, 2010) and since then, additional potential pharmacological targets such as the multi-subunit complex WRAD, which regulates the degree of H3K4 methylation by the MLL1 core complex, have emerged (Patel et al., 2009; Patel et al., 2011) (Figure 8).

In conclusion, this chapter summarizes recent biochemical studies that have contributed significantly to our understanding of how MLL1 works and have led to the identification of promising therapeutic targets for MLL1-related leukemias. In particular, inhibitors that target molecular interactions between menin and MLL1, AF4 and AF9, and MLL1 and WRAD have emerged as novel candidate small molecule targets. These MLL1-targeted therapies have enhanced pharmacological potential as compared to the existing broadly-based chemotherapeutics for MLL1-associated leukemias and will hopefully result in better treatment outcomes. In the future, similar biochemical, structural and genetic studies will be instrumental in identifying additional molecular targets that will form the basis for novel treatment strategies.

8. References

- Abramovich, C., and Humphries, R.K. (2005). Hox regulation of normal and leukemic hematopoietic stem cells. *Curr Opin Hematol* 12, 210-216.
- Adamson, A.L., and Shearn, A. (1996). Molecular genetic analysis of *Drosophila ash2*, a member of the trithorax group required for imaginal disc pattern formation. *Genetics* 144, 621-633.
- Agarwal, S.K., Guru, S.C., Heppner, C., Erdos, M.R., Collins, R.M., Park, S.Y., Saggari, S., Chandrasekharappa, S.C., Collins, F.S., Spiegel, A.M., *et al.* (1999). Menin interacts with the AP1 transcription factor JunD and represses JunD-activated transcription. *Cell* 96, 143-152.

- Agarwal, S.K., Novotny, E.A., Crabtree, J.S., Weitzman, J.B., Yaniv, M., Burns, A.L., Chandrasekharappa, S.C., Collins, F.S., Spiegel, A.M., and Marx, S.J. (2003). Transcription factor JunD, deprived of menin, switches from growth suppressor to growth promoter. *Proc Natl Acad Sci U S A* 100, 10770-10775.
- Allen, M.D., Grummitt, C.G., Hilcenko, C., Min, S.Y., Tonkin, L.M., Johnson, C.M., Freund, S.M., Bycroft, M., and Warren, A.J. (2006). Solution structure of the nonmethyl-CpG-binding CXXC domain of the leukaemia-associated MLL histone methyltransferase. *Embo J* 25, 4503-4512.
- Allen, R.J., Smith, S.D., Moldwin, R.L., Lu, M.M., Giordano, L., Vignon, C., Suto, Y., Harden, A., Tomek, R., Veldman, T., *et al.* (1998). Establishment and characterization of a megakaryoblast cell line with amplification of MLL. *Leukemia* 12, 1119-1127.
- Altucci, L., Clarke, N., Nebbioso, A., Scognamiglio, A., and Gronemeyer, H. (2005). Acute myeloid leukemia: therapeutic impact of epigenetic drugs. *Int J Biochem Cell Biol* 37, 1752-1762.
- Ansari, K.I., and Mandal, S.S. (2010). Mixed lineage leukemia: roles in gene expression, hormone signaling and mRNA processing. *Febs J* 277, 1790-1804.
- Ariyama, Y., Fukuda, Y., Okuno, Y., Seto, M., Date, K., Abe, T., Nakamura, Y., and Inazawa, J. (1998). Amplification on double-minute chromosomes and partial-tandem duplication of the MLL gene in leukemic cells of a patient with acute myelogenous leukemia. *Genes Chromosomes Cancer* 23, 267-272.
- Armstrong, S.A., Kung, A.L., Mabon, M.E., Silverman, L.B., Stam, R.W., Den Boer, M.L., Pieters, R., Kersey, J.H., Sallan, S.E., Fletcher, J.A., *et al.* (2003). Inhibition of FLT3 in MLL. Validation of a therapeutic target identified by gene expression based classification. *Cancer Cell* 3, 173-183.
- Armstrong, S.A., Staunton, J.E., Silverman, L.B., Pieters, R., den Boer, M.L., Minden, M.D., Sallan, S.E., Lander, E.S., Golub, T.R., and Korsmeyer, S.J. (2002). MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. *Nat Genet* 30, 41-47.
- Avet-Loiseau, H., Godon, C., Li, J.Y., Daviet, A., Mellerin, M.P., Talmant, P., Harousseau, J.L., and Bataille, R. (1999). Amplification of the 11q23 region in acute myeloid leukemia. *Genes Chromosomes Cancer* 26, 166-170.
- Ayton, P.M., Chen, E.H., and Cleary, M.L. (2004). Binding to nonmethylated CpG DNA is essential for target recognition, transactivation, and myeloid transformation by an MLL oncoprotein. *Mol Cell Biol* 24, 10470-10478.
- Ayton, P.M., and Cleary, M.L. (2003). Transformation of myeloid progenitors by MLL oncoproteins is dependent on Hoxa7 and Hoxa9. *Genes Dev* 17, 2298-2307.
- Balogh, K., Racz, K., Patocs, A., and Hunyady, L. (2006). Menin and its interacting proteins: elucidation of menin function. *Trends Endocrinol Metab* 17, 357-364.
- Basecke, J., Whelan, J.T., Griesinger, F., and Bertrand, F.E. (2006). The MLL partial tandem duplication in acute myeloid leukaemia. *Br J Haematol* 135, 438-449.
- Behm, F.G., Raimondi, S.C., Frestedt, J.L., Liu, Q., Crist, W.M., Downing, J.R., Rivera, G.K., Kersey, J.H., and Pui, C.H. (1996). Rearrangement of the MLL gene confers a poor prognosis in childhood acute lymphoblastic leukemia, regardless of presenting age. *Blood* 87, 2870-2877.
- Benedikt, A., Baltruschat, S., Scholz, B., Bursen, A., Arrey, T.N., Meyer, B., Varagnolo, L., Muller, A.M., Karas, M., Dingermann, T., *et al.* (2011). The leukemogenic AF4-MLL

- fusion protein causes P-TEFb kinase activation and altered epigenetic signatures. *Leukemia* 25, 135-144.
- Bennett, C.A., Winters, A.C., Barretto, N.N., and Hemenway, C.S. (2009). Molecular targeting of MLL-rearranged leukemia cell lines with the synthetic peptide PFWT synergistically enhances the cytotoxic effect of established chemotherapeutic agents. *Leuk Res* 33, 937-947.
- Bensaid, M., Melko, M., Bechara, E.G., Davidovic, L., Berretta, A., Catania, M.V., Gecz, J., Lalli, E., and Bardoni, B. (2009). FRAXE-associated mental retardation protein (FMR2) is an RNA-binding protein with high affinity for G-quartet RNA forming structure. *Nucleic Acids Res* 37, 1269-1279.
- Bernard, O.A., Mauchauffe, M., Mecucci, C., Van den Berghe, H., and Berger, R. (1994). A novel gene, AF-1p, fused to HRX in t(1;11)(p32;q23), is not related to AF-4, AF-9 nor ENL. *Oncogene* 9, 1039-1045.
- Bernstein, B.E., Humphrey, E.L., Erlich, R.L., Schneider, R., Bouman, P., Liu, J.S., Kouzarides, T., and Schreiber, S.L. (2002). Methylation of histone H3 Lys 4 in coding regions of active genes. *Proc Natl Acad Sci U S A* 99, 8695-8700.
- Bernstein, B.E., Kamal, M., Lindblad-Toh, K., Bekiranov, S., Bailey, D.K., Huebert, D.J., McMahon, S., Karlsson, E.K., Kulbokas, E.J., 3rd, Gingeras, T.R., *et al.* (2005). Genomic maps and comparative analysis of histone modifications in human and mouse. *Cell* 120, 169-181.
- Bertolino, P., Radovanovic, I., Casse, H., Aguzzi, A., Wang, Z.Q., and Zhang, C.X. (2003a). Genetic ablation of the tumor suppressor menin causes lethality at mid-gestation with defects in multiple organs. *Mech Dev* 120, 549-560.
- Bertolino, P., Tong, W.M., Galendo, D., Wang, Z.Q., and Zhang, C.X. (2003b). Heterozygous Men1 mutant mice develop a range of endocrine tumors mimicking multiple endocrine neoplasia type 1. *Mol Endocrinol* 17, 1880-1892.
- Bigoni, R., Cuneo, A., Roberti, M.G., Moretti, S., De Angeli, C., Dabusti, M., Campioni, D., del Senno, L., Biondi, A., Chaplin, T., *et al.* (1999). Therapy-related adult acute lymphoblastic leukemia with t(4;11)(q21; q23): MLL rearrangement, p53 mutation and multilineage involvement. *Leukemia* 13, 704-707.
- Bitoun, E., Oliver, P.L., and Davies, K.E. (2007). The mixed-lineage leukemia fusion partner AF4 stimulates RNA polymerase II transcriptional elongation and mediates coordinated chromatin remodeling. *Hum Mol Genet* 16, 92-106.
- Boggs, B.A., Cheung, P., Heard, E., Spector, D.L., Chinault, A.C., and Allis, C.D. (2002). Differentially methylated forms of histone H3 show unique association patterns with inactive human X chromosomes. *Nat Genet* 30, 73-76.
- Bralten, L.B., Kloosterhof, N.K., Gravendeel, L.A., Sacchetti, A., Duijm, E.J., Kros, J.M., van den Bent, M.J., Hoogenraad, C.C., Sillevius Smitt, P.A., and French, P.J. (2010). Integrated genomic profiling identifies candidate genes implicated in gliomagenesis and a novel LEO1-SLC12A1 fusion gene. *Genes Chromosomes Cancer* 49, 509-517.
- Brown, P., Levis, M., Shurtleff, S., Campana, D., Downing, J., and Small, D. (2005). FLT3 inhibition selectively kills childhood acute lymphoblastic leukemia cells with high levels of FLT3 expression. *Blood* 105, 812-820.
- Burmeister, T., Meyer, C., Schwartz, S., Hofmann, J., Molkenin, M., Kowarz, E., Schneider, B., Raff, T., Reinhardt, R., Gokbuget, N., *et al.* (2009). The MLL recombinome of

- adult CD10-negative B-cell precursor acute lymphoblastic leukemia: results from the GMALL study group. *Blood* 113, 4011-4015.
- Bursen, A., Schwabe, K., Ruster, B., Henschler, R., Ruthardt, M., Dingermann, T., and Marschalek, R. (2010). The AF4.MLL fusion protein is capable of inducing ALL in mice without requirement of MLL.AF4. *Blood* 115, 3570-3579.
- Cairns, B.R., Henry, N.L., and Kornberg, R.D. (1996). TFG/TAF30/ANC1, a component of the yeast SWI/SNF complex that is similar to the leukemogenic proteins ENL and AF-9. *Mol Cell Biol* 16, 3308-3316.
- Caligiuri, M.A., Schichman, S.A., Strout, M.P., Mrozek, K., Baer, M.R., Frankel, S.R., Barcos, M., Herzig, G.P., Croce, C.M., and Bloomfield, C.D. (1994). Molecular rearrangement of the ALL-1 gene in acute myeloid leukemia without cytogenetic evidence of 11q23 chromosomal translocations. *Cancer Res* 54, 370-373.
- Caligiuri, M.A., Strout, M.P., Lawrence, D., Arthur, D.C., Baer, M.R., Yu, F., Knuutila, S., Mrozek, K., Oberkircher, A.R., Marcucci, G., *et al.* (1998). Rearrangement of ALL1 (MLL) in acute myeloid leukemia with normal cytogenetics. *Cancer Res* 58, 55-59.
- Campo Dell'Orto, M., Banelli, B., Giarin, E., Accordi, B., Trentin, L., Romani, M., te Kronnie, G., and Basso, G. (2007). Down-regulation of DLX3 expression in MLL-AF4 childhood lymphoblastic leukemias is mediated by promoter region hypermethylation. *Oncol Rep* 18, 417-423.
- Carlson, M., and Laurent, B.C. (1994). The SNF/SWI family of global transcriptional activators. *Curr Opin Cell Biol* 6, 396-402.
- Carnicer, M.J., Nomdedeu, J.F., Lasa, A., Estivill, C., Brunet, S., Aventin, A., and Sierra, J. (2004). FLT3 mutations are associated with other molecular lesions in AML. *Leuk Res* 28, 19-23.
- Caslini, C., Yang, Z., El-Osta, M., Milne, T.A., Slany, R.K., and Hess, J.L. (2007). Interaction of MLL amino terminal sequences with menin is required for transformation. *Cancer Res* 67, 7275-7283.
- Chandrasekharappa, S.C., Guru, S.C., Manickam, P., Olufemi, S.E., Collins, F.S., Emmert-Buck, M.R., Debelenko, L.V., Zhuang, Z., Lubensky, I.A., Liotta, L.A., *et al.* (1997). Positional cloning of the gene for multiple endocrine neoplasia-type 1. *Science* 276, 404-407.
- Chandrasekharappa, S.C., and Teh, B.T. (2001). Clinical and molecular aspects of multiple endocrine neoplasia type 1. *Front Horm Res* 28, 50-80.
- Chandrasekharappa, S.C., and Teh, B.T. (2003). Functional studies of the MEN1 gene. *J Intern Med* 253, 606-615.
- Chen, C.S., Hilden, J.M., Frestedt, J., Domer, P.H., Moore, R., Korsmeyer, S.J., and Kersey, J.H. (1993). The chromosome 4q21 gene (AF-4/FEL) is widely expressed in normal tissues and shows breakpoint diversity in t(4;11)(q21;q23) acute leukemia. *Blood* 82, 1080-1085.
- Chen, R., Wierda, W.G., Chubb, S., Hawtin, R.E., Fox, J.A., Keating, M.J., Gandhi, V., and Plunkett, W. (2009). Mechanism of action of SNS-032, a novel cyclin-dependent kinase inhibitor, in chronic lymphocytic leukemia. *Blood* 113, 4637-4645.
- Chen, W., Li, Q., Hudson, W.A., Kumar, A., Kirchhof, N., and Kersey, J.H. (2006a). A murine Mll-AF4 knock-in model results in lymphoid and myeloid deregulation and hematologic malignancy. *Blood* 108, 669-677.

- Chen, Y.X., Yan, J., Keeshan, K., Tubbs, A.T., Wang, H., Silva, A., Brown, E.J., Hess, J.L., Pear, W.S., and Hua, X. (2006b). The tumor suppressor menin regulates hematopoiesis and myeloid transformation by influencing Hox gene expression. *Proc Natl Acad Sci U S A* 103, 1018-1023.
- Cheng, X., Collins, R.E., and Zhang, X. (2005). Structural and sequence motifs of protein (histone) methylation enzymes. *Annu Rev Biophys Biomol Struct* 34, 267-294.
- Cheung, N., Chan, L.C., Thompson, A., Cleary, M.L., and So, C.W. (2007). Protein arginine-methyltransferase-dependent oncogenesis. *Nat Cell Biol* 9, 1208-1215.
- Cho, Y.W., Hong, T., Hong, S., Guo, H., Yu, H., Kim, D., Guszczynski, T., Dressler, G.R., Copeland, T.D., Kalkum, M., *et al.* (2007). PTIP associates with MLL3- and MLL4-containing histone H3 lysine 4 methyltransferase complex. *J Biol Chem* 282, 20395-20406.
- Cierpicki, T., Risner, L.E., Grembecka, J., Lukasik, S.M., Popovic, R., Omonkowska, M., Shultis, D.D., Zeleznik-Le, N.J., and Bushweller, J.H. (2010). Structure of the MLL CXXC domain-DNA complex and its functional role in MLL-AF9 leukemia. *Nat Struct Mol Biol* 17, 62-68.
- Cohen, A., Grunberger, T., Vanek, W., Dube, I.D., Doherty, P.J., Letarte, M., Roifman, C., and Freedman, M.H. (1991). Constitutive expression and role in growth regulation of interleukin-1 and multiple cytokine receptors in a biphenotypic leukemic cell line. *Blood* 78, 94-102.
- Collins, E.C., Appert, A., Ariza-McNaughton, L., Pannell, R., Yamada, Y., and Rabbitts, T.H. (2002). Mouse Af9 is a controller of embryo patterning, like Mll, whose human homologue fuses with Af9 after chromosomal translocation in leukemia. *Mol Cell Biol* 22, 7313-7324.
- Collins, R.E., Tachibana, M., Tamaru, H., Smith, K.M., Jia, D., Zhang, X., Selker, E.U., Shinkai, Y., and Cheng, X. (2005). In vitro and in vivo analyses of a Phe/Tyr switch controlling product specificity of histone lysine methyltransferases. *J Biol Chem* 280, 5563-5570.
- Cosgrove, M.S., and Patel, A. (2010). Mixed lineage leukemia: a structure-function perspective of the MLL1 protein. *Febs Journal* 277, 1832-1842.
- Cosgrove, M.S., and Wolberger, C. (2005). How does the histone code work? *Biochem Cell Biol* 83, 468-476.
- Cote, J., Quinn, J., Workman, J.L., and Peterson, C.L. (1994). Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science* 265, 53-60.
- Couture, J.F., Collazo, E., and Trievel, R.C. (2006). Molecular recognition of histone H3 by the WD40 protein WDR5. *Nat Struct Mol Biol* 13, 698-703.
- Crabtree, J.S., Scacheri, P.C., Ward, J.M., Garrett-Beal, L., Emmert-Buck, M.R., Edgemon, K.A., Lorang, D., Libutti, S.K., Chandrasekharappa, S.C., Marx, S.J., *et al.* (2001). A mouse model of multiple endocrine neoplasia, type 1, develops multiple endocrine tumors. *Proc Natl Acad Sci U S A* 98, 1118-1123.
- Cuthbert, G., McCullough, S., Finney, R., Breese, G., and Bown, N. (1999). Jumping translocation at 11q23 with MLL gene rearrangement and interstitial telomeric sequences. *Genes Chromosomes Cancer* 24, 295-298.
- Daser, A., and Rabbitts, T.H. (2005). The versatile mixed lineage leukaemia gene MLL and its many associations in leukaemogenesis. *Semin Cancer Biol* 15, 175-188.

- Debernardi, S., Bassini, A., Jones, L.K., Chaplin, T., Linder, B., de Bruijn, D.R., Meese, E., and Young, B.D. (2002). The MLL fusion partner AF10 binds GAS41, a protein that interacts with the human SWI/SNF complex. *Blood* 99, 275-281.
- Dillon, S.C., Zhang, X., Trievel, R.C., and Cheng, X. (2005). The SET-domain protein superfamily: protein lysine methyltransferases. *Genome Biol* 6, 227.
- Djabali, M., Selleri, L., Parry, P., Bower, M., Young, B.D., and Evans, G.A. (1992). A trithorax-like gene is interrupted by chromosome 11q23 translocations in acute leukaemias. *Nat Genet* 2, 113-118.
- Dobson, C.L., Warren, A.J., Pannell, R., Forster, A., Lavenir, I., Corral, J., Smith, A.J., and Rabbitts, T.H. (1999). The mll-AF9 gene fusion in mice controls myeloproliferation and specifies acute myeloid leukaemogenesis. *Embo J* 18, 3564-3574.
- Dobson, C.L., Warren, A.J., Pannell, R., Forster, A., and Rabbitts, T.H. (2000). Tumorigenesis in mice with a fusion of the leukaemia oncogene Mll and the bacterial lacZ gene. *Embo J* 19, 843-851.
- Dohner, K., Tobis, K., Ulrich, R., Frohling, S., Benner, A., Schlenk, R.F., and Dohner, H. (2002). Prognostic significance of partial tandem duplications of the MLL gene in adult patients 16 to 60 years old with acute myeloid leukemia and normal cytogenetics: a study of the Acute Myeloid Leukemia Study Group Ulm. *J Clin Oncol* 20, 3254-3261.
- Domer, P.H., Fakharzadeh, S.S., Chen, C.S., Jockel, J., Johansen, L., Silverman, G.A., Kersey, J.H., and Korsmeyer, S.J. (1993). Acute mixed-lineage leukemia t(4;11)(q21;q23) generates an MLL-AF4 fusion product. *Proc Natl Acad Sci U S A* 90, 7884-7888.
- Dong, Q., Debelenko, L.V., Chandrasekharappa, S.C., Emmert-Buck, M.R., Zhuang, Z., Guru, S.C., Manickam, P., Skarulis, M., Lubensky, I.A., Liotta, L.A., *et al.* (1997). Loss of heterozygosity at 11q13: analysis of pituitary tumors, lung carcinoids, lipomas, and other uncommon tumors in subjects with familial multiple endocrine neoplasia type 1. *J Clin Endocrinol Metab* 82, 1416-1420.
- Dorrance, A.M., Liu, S., Chong, A., Pulley, B., Nemer, D., Guimond, M., Yuan, W., Chang, D., Whitman, S.P., Marcucci, G., *et al.* (2008). The Mll partial tandem duplication: differential, tissue-specific activity in the presence or absence of the wild-type allele. *Blood* 112, 2508-2511.
- Dorrance, A.M., Liu, S., Yuan, W., Becknell, B., Arnoczky, K.J., Guimond, M., Strout, M.P., Feng, L., Nakamura, T., Yu, L., *et al.* (2006). Mll partial tandem duplication induces aberrant Hox expression in vivo via specific epigenetic alterations. *J Clin Invest* 116, 2707-2716.
- Doty, R.T., Vanasse, G.J., Disteche, C.M., and Willerford, D.M. (2002). The leukemia-associated gene Mllt1/ENL: characterization of a murine homolog and demonstration of an essential role in embryonic development. *Blood Cells Mol Dis* 28, 407-417.
- Dou, Y., and Hess, J.L. (2008). Mechanisms of transcriptional regulation by MLL and its disruption in acute leukemia. *Int J Hematol* 87, 10-18.
- Dou, Y., Milne, T.A., Ruthenburg, A.J., Lee, S., Lee, J.W., Verdine, G.L., Allis, C.D., and Roeder, R.G. (2006). Regulation of MLL1 H3K4 methyltransferase activity by its core components. *Nat Struct Mol Biol* 13, 713-719.
- Dou, Y., Milne, T.A., Tackett, A.J., Smith, E.R., Fukuda, A., Wysocka, J., Allis, C.D., Chait, B.T., Hess, J.L., and Roeder, R.G. (2005). Physical association and coordinate

- function of the H3 K4 methyltransferase MLL1 and the H4 K16 acetyltransferase MOF. *Cell* 121, 873-885.
- Downing, J.R. (2008). Targeted therapy in leukemia. *Mod Pathol* 21 Suppl 2, S2-7.
- Dreijerink, K.M., Mulder, K.W., Winkler, G.S., Hoppener, J.W., Lips, C.J., and Timmers, H.T. (2006). Menin links estrogen receptor activation to histone H3K4 trimethylation. *Cancer Res* 66, 4929-4935.
- Eguchi, M., Eguchi-Ishimae, M., and Greaves, M. (2004). The small oligomerization domain of gephyrin converts MLL to an oncogene. *Blood* 103, 3876-3882.
- Erfurth, F., Hemenway, C.S., de Erkenez, A.C., and Domer, P.H. (2004). MLL fusion partners AF4 and AF9 interact at subnuclear foci. *Leukemia* 18, 92-102.
- Ernst, P., Fisher, J.K., Avery, W., Wade, S., Foy, D., and Korsmeyer, S.J. (2004a). Definitive hematopoiesis requires the mixed-lineage leukemia gene. *Dev Cell* 6, 437-443.
- Ernst, P., Mabon, M., Davidson, A.J., Zon, L.I., and Korsmeyer, S.J. (2004b). An Mll-dependent Hox program drives hematopoietic progenitor expansion. *Curr Biol* 14, 2063-2069.
- Ernst, P., Wang, J., Huang, M., Goodman, R.H., and Korsmeyer, S.J. (2001). MLL and CREB bind cooperatively to the nuclear coactivator CREB-binding protein. *Mol Cell Biol* 21, 2249-2258.
- Ernst, P., Wang, J., and Korsmeyer, S.J. (2002). The role of MLL in hematopoiesis and leukemia. *Curr Opin Hematol* 9, 282-287.
- Estable, M.C., Naghavi, M.H., Kato, H., Xiao, H., Qin, J., Vahlne, A., and Roeder, R.G. (2002). MCEF, the newest member of the AF4 family of transcription factors involved in leukemia, is a positive transcription elongation factor-b-associated protein. *J Biomed Sci* 9, 234-245.
- Faderl, S., Kantarjian, H.M., Talpaz, M., and Estrov, Z. (1998). Clinical significance of cytogenetic abnormalities in adult acute lymphoblastic leukemia. *Blood* 91, 3995-4019.
- Felix, C.A., Lange, B.J., Hosler, M.R., Fertala, J., and Bjornsti, M.A. (1995). Chromosome band 11q23 translocation breakpoints are DNA topoisomerase II cleavage sites. *Cancer Res* 55, 4287-4292.
- Felix, C.A., Megonigal, M.D., Chervinsky, D.S., Leonard, D.G., Tsuchida, N., Kakati, S., Block, A.M., Fisher, J., Grossi, M., Salhany, K.I., *et al.* (1998). Association of germline p53 mutation with MLL segmental jumping translocation in treatment-related leukemia. *Blood* 91, 4451-4456.
- Ferrando, A.A., Armstrong, S.A., Neuberg, D.S., Sallan, S.E., Silverman, L.B., Korsmeyer, S.J., and Look, A.T. (2003). Gene expression signatures in MLL-rearranged T-lineage and B-precursor acute leukemias: dominance of HOX dysregulation. *Blood* 102, 262-268.
- Flanagan, J.F., Mi, L.Z., Chruszcz, M., Cymborowski, M., Clines, K.L., Kim, Y., Minor, W., Rastinejad, F., and Khorasanizadeh, S. (2005). Double chromodomains cooperate to recognize the methylated histone H3 tail. *Nature* 438, 1181-1185.
- Gan, T., Jude, C.D., Zaffuto, K., and Ernst, P. (2010). Developmentally induced Mll1 loss reveals defects in postnatal haematopoiesis. *Leukemia* 24, 1732-1741.
- Ganapathy, V., Gopal, E., Miyauchi, S., and Prasad, P.D. (2005). Biological functions of SLC5A8, a candidate tumour suppressor. *Biochem Soc Trans* 33, 237-240.

- Gobl, A.E., Berg, M., Lopez-Egido, J.R., Oberg, K., Skogseid, B., and Westin, G. (1999). Menin represses JunD-activated transcription by a histone deacetylase-dependent mechanism. *Biochim Biophys Acta* 1447, 51-56.
- Gori, F., and Demay, M.B. (2005). The effects of BIG-3 on osteoblast differentiation are not dependent upon endogenously produced BMPs. *Exp Cell Res* 304, 287-292.
- Gori, F., Friedman, L., and Demay, M.B. (2005). Wdr5, a novel WD repeat protein, regulates osteoblast and chondrocyte differentiation in vivo. *J Musculoskelet Neuronal Interact* 5, 338-339.
- Grembecka, J., Belcher, A.M., Hartley, T., and Cierpicki, T. (2010). Molecular basis of the mixed lineage leukemia-menin interaction: implications for targeting mixed lineage leukemias. *J Biol Chem* 285, 40690-40698.
- Gu, Y., Nakamura, T., Alder, H., Prasad, R., Canaani, O., Cimino, G., Croce, C.M., and Canaani, E. (1992). The t(4;11) chromosome translocation of human acute leukemias fuses the ALL-1 gene, related to *Drosophila trithorax*, to the AF-4 gene. *Cell* 71, 701-708.
- Gu, Y., Shen, Y., Gibbs, R.A., and Nelson, D.L. (1996). Identification of FMR2, a novel gene associated with the FRAXE CCG repeat and CpG island. *Nat Genet* 13, 109-113.
- Gupta, N., Martin, P.M., Prasad, P.D., and Ganapathy, V. (2006). SLC5A8 (SMCT1)-mediated transport of butyrate forms the basis for the tumor suppressive function of the transporter. *Life Sci* 78, 2419-2425.
- Guru, S.C., Crabtree, J.S., Brown, K.D., Dunn, K.J., Manickam, P., Prasad, N.B., Wangsa, D., Burns, A.L., Spiegel, A.M., Marx, S.J., *et al.* (1999). Isolation, genomic organization, and expression analysis of Men1, the murine homolog of the MEN1 gene. *Mamm Genome* 10, 592-596.
- Guru, S.C., Goldsmith, P.K., Burns, A.L., Marx, S.J., Spiegel, A.M., Collins, F.S., and Chandrasekharappa, S.C. (1998). Menin, the product of the MEN1 gene, is a nuclear protein. *Proc Natl Acad Sci U S A* 95, 1630-1634.
- Guru, S.C., Prasad, N.B., Shin, E.J., Hemavathy, K., Lu, J., Ip, Y.T., Agarwal, S.K., Marx, S.J., Spiegel, A.M., Collins, F.S., *et al.* (2001). Characterization of a MEN1 ortholog from *Drosophila melanogaster*. *Gene* 263, 31-38.
- Hall, P.A., and Russell, S.E. (2004). The pathobiology of the septin gene family. *J Pathol* 204, 489-505.
- Han, Z., Guo, L., Wang, H., Shen, Y., Deng, X.W., and Chai, J. (2006). Structural basis for the specific recognition of methylated histone H3 lysine 4 by the WD-40 protein WDR5. *Mol Cell* 22, 137-144.
- Hanson, R.D., Hess, J.L., Yu, B.D., Ernst, P., van Lohuizen, M., Berns, A., van der Lugt, N.M., Shashikant, C.S., Ruddle, F.H., Seto, M., *et al.* (1999). Mammalian Trithorax and polycomb-group homologues are antagonistic regulators of homeotic development. *Proc Natl Acad Sci U S A* 96, 14372-14377.
- Heerema, N.A., Sather, H.N., Ge, J., Arthur, D.C., Hilden, J.M., Trigg, M.E., and Reaman, G.H. (1999). Cytogenetic studies of infant acute lymphoblastic leukemia: poor prognosis of infants with t(4;11) - a report of the Children's Cancer Group. *Leukemia* 13, 679-686.
- Heintzman, N.D., Stuart, R.K., Hon, G., Fu, Y., Ching, C.W., Hawkins, R.D., Barrera, L.O., Van Calcar, S., Qu, C., Ching, K.A., *et al.* (2007). Distinct and predictive chromatin

- signatures of transcriptional promoters and enhancers in the human genome. *Nat Genet* 39, 311-318.
- Hemenway, C.S., de Erkenez, A.C., and Gould, G.C. (2001). The polycomb protein MPC3 interacts with AF9, an MLL fusion partner in t(9;11)(p22;q23) acute leukemias. *Oncogene* 20, 3798-3805.
- Heppner, C., Bilimoria, K.Y., Agarwal, S.K., Kester, M., Whitty, L.J., Guru, S.C., Chandrasekharappa, S.C., Collins, F.S., Spiegel, A.M., Marx, S.J., *et al.* (2001). The tumor suppressor protein menin interacts with NF-kappaB proteins and inhibits NF-kappaB-mediated transactivation. *Oncogene* 20, 4917-4925.
- Herry, A., Douet-Guilbert, N., Gueganic, N., Morel, F., Le Bris, M.J., Berthou, C., and De Braekeleer, M. (2006). Del(5q) and MLL amplification in homogeneously staining region in acute myeloblastic leukemia: a recurrent cytogenetic association. *Ann Hematol* 85, 244-249.
- Hess, J.L. (2004). MLL: a histone methyltransferase disrupted in leukemia. *Trends Mol Med* 10, 500-507.
- Hess, J.L., Bittner, C.B., Zeisig, D.T., Bach, C., Fuchs, U., Borkhardt, A., Frampton, J., and Slany, R.K. (2006). c-Myb is an essential downstream target for homeobox-mediated transformation of hematopoietic cells. *Blood* 108, 297-304.
- Hess, J.L., Yu, B.D., Li, B., Hanson, R., and Korsmeyer, S.J. (1997). Defects in yolk sac hematopoiesis in Mll-null embryos. *Blood* 90, 1799-1806.
- Hsieh, J.J., Cheng, E.H., and Korsmeyer, S.J. (2003a). Taspase1: a threonine aspartase required for cleavage of MLL and proper HOX gene expression. *Cell* 115, 293-303.
- Hsieh, J.J., Ernst, P., Erdjument-Bromage, H., Tempst, P., and Korsmeyer, S.J. (2003b). Proteolytic cleavage of MLL generates a complex of N- and C-terminal fragments that confers protein stability and subnuclear localization. *Mol Cell Biol* 23, 186-194.
- Hsu, D.R., Chuang, P.T., and Meyer, B.J. (1995). DPY-30, a nuclear protein essential early in embryogenesis for *Caenorhabditis elegans* dosage compensation. *Development* 121, 3323-3334.
- Hsu, D.R., and Meyer, B.J. (1994). The dpy-30 gene encodes an essential component of the *Caenorhabditis elegans* dosage compensation machinery. *Genetics* 137, 999-1018.
- Hughes, C.M., Rozenblatt-Rosen, O., Milne, T.A., Copeland, T.D., Levine, S.S., Lee, J.C., Hayes, D.N., Shanmugam, K.S., Bhattacharjee, A., Biondi, C.A., *et al.* (2004). Menin associates with a trithorax family histone methyltransferase complex and with the *hoxc8* locus. *Mol Cell* 13, 587-597.
- Huret, J.L., Dessen, P., and Bernheim, A. (2001). An atlas of chromosomes in hematological malignancies. Example: 11q23 and MLL partners. *Leukemia* 15, 987-989.
- Ida, K., Kitabayashi, I., Taki, T., Taniwaki, M., Noro, K., Yamamoto, M., Ohki, M., and Hayashi, Y. (1997). Adenoviral E1A-associated protein p300 is involved in acute myeloid leukemia with t(11;22)(q23;q13). *Blood* 90, 4699-4704.
- Isnard, P., Core, N., Naquet, P., and Djabali, M. (2000). Altered lymphoid development in mice deficient for the mAF4 proto-oncogene. *Blood* 96, 705-710.
- Jin, S., Mao, H., Schnepf, R.W., Sykes, S.M., Silva, A.C., D'Andrea, A.D., and Hua, X. (2003). Menin associates with FANCD2, a protein involved in repair of DNA damage. *Cancer Res* 63, 4204-4210.

- Jin, S., Zhao, H., Yi, Y., Nakata, Y., Kalota, A., and Gewirtz, A.M. (2010). c-Myb binds MLL through menin in human leukemia cells and is an important driver of MLL-associated leukemogenesis. *J Clin Invest* 120, 593-606.
- Jude, C.D., Climer, L., Xu, D., Artinger, E., Fisher, J.K., and Ernst, P. (2007). Unique and independent roles for MLL in adult hematopoietic stem cells and progenitors. *Cell Stem Cell* 1, 324-337.
- Kaji, H., Canaff, L., Lebrun, J.J., Goltzman, D., and Hendy, G.N. (2001). Inactivation of menin, a Smad3-interacting protein, blocks transforming growth factor type beta signaling. *Proc Natl Acad Sci U S A* 98, 3837-3842.
- Karatas, H., Townsend, E.C., Bernard, D., Dou, Y., and Wang, S. (2010). Analysis of the binding of mixed lineage leukemia 1 (MLL1) and histone 3 peptides to WD repeat domain 5 (WDR5) for the design of inhibitors of the MLL1-WDR5 interaction. *J Med Chem* 53, 5179-5185.
- Khodaei, S., O'Brien, K.P., Dumanski, J., Wong, F.K., and Weber, G. (1999). Characterization of the MEN1 ortholog in zebrafish. *Biochem Biophys Res Commun* 264, 404-408.
- Koziner, B., Stavnezer, J., and Al-Katib, A. (1985). Surface immunoglobulin light-chain expression by the "common" all cell line REH. *Clin Immunol Immunopathol* 37, 135-141.
- Krivtsov, A.V., and Armstrong, S.A. (2007). MLL translocations, histone modifications and leukaemia stem-cell development. *Nat Rev Cancer* 7, 823-833.
- Kroon, E., Kros, J., Thorsteinsdottir, U., Baban, S., Buchberg, A.M., and Sauvageau, G. (1998). Hoxa9 transforms primary bone marrow cells through specific collaboration with Meis1a but not Pbx1b. *Embo J* 17, 3714-3725.
- Kumar, A.R., Hudson, W.A., Chen, W., Nishiuchi, R., Yao, Q., and Kersey, J.H. (2004). Hoxa9 influences the phenotype but not the incidence of MLL-AF9 fusion gene leukemia. *Blood* 103, 1823-1828.
- La, P., Schnepf, R.W., C, D.P., A, C.S., and Hua, X. (2004). Tumor suppressor menin regulates expression of insulin-like growth factor binding protein 2. *Endocrinology* 145, 3443-3450.
- Lange, B., Valtieri, M., Santoli, D., Caracciolo, D., Mavilio, F., Gemperlein, I., Griffin, C., Emanuel, B., Finan, J., Nowell, P., *et al.* (1987). Growth factor requirements of childhood acute leukemia: establishment of GM-CSF-dependent cell lines. *Blood* 70, 192-199.
- Larsson, C., Skogseid, B., Oberg, K., Nakamura, Y., and Nordenskjold, M. (1988). Multiple endocrine neoplasia type 1 gene maps to chromosome 11 and is lost in insulinoma. *Nature* 332, 85-87.
- Lavau, C., Du, C., Thirman, M., and Zeleznik-Le, N. (2000). Chromatin-related properties of CBP fused to MLL generate a myelodysplastic-like syndrome that evolves into myeloid leukemia. *Embo J* 19, 4655-4664.
- Lee, J.H., and Skalnik, D.G. (2005). CpG-binding protein (CXXC finger protein 1) is a component of the mammalian Set1 histone H3-Lys4 methyltransferase complex, the analogue of the yeast Set1/COMPASS complex. *J Biol Chem* 280, 41725-41731.
- Lee, J.H., Tate, C.M., You, J.S., and Skalnik, D.G. (2007). Identification and characterization of the human Set1B histone H3-Lys4 methyltransferase complex. *J Biol Chem* 282, 13419-13428.

- Lemmens, I., Van de Ven, W.J., Kas, K., Zhang, C.X., Giraud, S., Wautot, V., Buisson, N., De Witte, K., Salandre, J., Lenoir, G., *et al.* (1997). Identification of the multiple endocrine neoplasia type 1 (MEN1) gene. The European Consortium on MEN1. *Hum Mol Genet* 6, 1177-1183.
- Lemmens, I.H., Forsberg, L., Pannett, A.A., Meyen, E., Piehl, F., Turner, J.J., Van de Ven, W.J., Thakker, R.V., Larsson, C., and Kas, K. (2001). Menin interacts directly with the homeobox-containing protein Pem. *Biochem Biophys Res Commun* 286, 426-431.
- Liang, D.C., Shih, L.Y., Fu, J.F., Li, H.Y., Wang, H.I., Hung, I.J., Yang, C.P., Jaing, T.H., Chen, S.H., and Liu, H.C. (2006). K-Ras mutations and N-Ras mutations in childhood acute leukemias with or without mixed-lineage leukemia gene rearrangements. *Cancer* 106, 950-956.
- Liedtke, M., and Cleary, M.L. (2009). Therapeutic targeting of MLL. *Blood* 113, 6061-6068.
- Litt, M.D., Simpson, M., Gaszner, M., Allis, C.D., and Felsenfeld, G. (2001). Correlation between histone lysine methylation and developmental changes at the chicken beta-globin locus. *Science* 293, 2453-2455.
- Liu, H., Cheng, E.H., and Hsieh, J.J. (2009). MLL fusions: pathways to leukemia. *Cancer Biol Ther* 8, 1204-1211.
- Lopez-Egido, J., Cunningham, J., Berg, M., Oberg, K., Bongcam-Rudloff, E., and Gobl, A. (2002). Menin's interaction with glial fibrillary acidic protein and vimentin suggests a role for the intermediate filament network in regulating menin activity. *Exp Cell Res* 278, 175-183.
- Luger, K., and Hansen, J.C. (2005). Nucleosome and chromatin fiber dynamics. *Curr Opin Struct Biol* 15, 188-196.
- Luo, R.T., Lavau, C., Du, C., Simone, F., Polak, P.E., Kawamata, S., and Thirman, M.J. (2001). The elongation domain of ELL is dispensable but its ELL-associated factor 1 interaction domain is essential for MLL-ELL-induced leukemogenesis. *Mol Cell Biol* 21, 5678-5687.
- Luscher-Firzloff, J., Gawlista, I., Vervoorts, J., Kapelle, K., Braunschweig, T., Walsemann, G., Rodgarkia-Schamberger, C., Schuchlantz, H., Dreschers, S., Kremmer, E., *et al.* (2008). The human trithorax protein hASH2 functions as an oncoprotein. *Cancer Res* 68, 749-758.
- Ma, C., and Staudt, L.M. (1996). LAF-4 encodes a lymphoid nuclear protein with transactivation potential that is homologous to AF-4, the gene fused to MLL in t(4;11) leukemias. *Blood* 87, 734-745.
- Macrini, C.M., Pombo-de-Oliveira, M.S., Ford, A.M., and Alves, G. (2003). MLL AT-hook sequence is strongly conserved in infant acute leukemia with or without MLL gene rearrangement. *Leukemia* 17, 1432-1433.
- Mahgoub, N., Parker, R.I., Hosler, M.R., Close, P., Winick, N.J., Masterson, M., Shannon, K.M., and Felix, C.A. (1998). RAS mutations in pediatric leukemias with MLL gene rearrangements. *Genes Chromosomes Cancer* 21, 270-275.
- Maillard, I., Chen, Y.X., Friedman, A., Yang, Y., Tubbs, A.T., Shestova, O., Pear, W.S., and Hua, X. (2009). Menin regulates the function of hematopoietic stem cells and lymphoid progenitors. *Blood* 113, 1661-1669.
- Maillard, I., and Hess, J.L. (2009). The role of menin in hematopoiesis. *Adv Exp Med Biol* 668, 51-57.

- Manickam, P., Vogel, A.M., Agarwal, S.K., Oda, T., Spiegel, A.M., Marx, S.J., Collins, F.S., Weinstein, B.M., and Chandrasekharappa, S.C. (2000). Isolation, characterization, expression and functional analysis of the zebrafish ortholog of MEN1. *Mamm Genome* 11, 448-454.
- Marschalek, R. (2010). Mixed lineage leukemia: roles in human malignancies and potential therapy. *Febs J* 277, 1822-1831.
- Martin, M.E., Milne, T.A., Bloyer, S., Galoian, K., Shen, W., Gibbs, D., Brock, H.W., Slany, R., and Hess, J.L. (2003). Dimerization of MLL fusion proteins immortalizes hematopoietic cells. *Cancer Cell* 4, 197-207.
- McMahon, K.A., Hiew, S.Y., Hadjur, S., Veiga-Fernandes, H., Menzel, U., Price, A.J., Kioussis, D., Williams, O., and Brady, H.J. (2007). Mll has a critical role in fetal and adult hematopoietic stem cell self-renewal. *Cell Stem Cell* 1, 338-345.
- Melko, M., Douguet, D., Bensaid, M., Zongaro, S., Verheggen, C., Gecz, J., and Bardoni, B. Functional characterization of the AFF (AF4/FMR2) family of RNA-binding proteins: insights into the molecular pathology of FRAXE intellectual disability. *Hum Mol Genet* 20, 1873-1885.
- Meyer, C., Kowarz, E., Hofmann, J., Renneville, A., Zuna, J., Trka, J., Ben Abdelali, R., Macintyre, E., De Braekeleer, E., De Braekeleer, M., *et al.* (2009). New insights to the MLL recombinome of acute leukemias. *Leukemia* 23, 1490-1499.
- Meyer, C., Schneider, B., Jakob, S., Strehl, S., Attarbaschi, A., Schnittger, S., Schoch, C., Jansen, M.W., van Dongen, J.J., den Boer, M.L., *et al.* (2006). The MLL recombinome of acute leukemias. *Leukemia* 20, 777-784.
- Milne, T.A., Briggs, S.D., Brock, H.W., Martin, M.E., Gibbs, D., Allis, C.D., and Hess, J.L. (2002). MLL targets SET domain methyltransferase activity to Hox gene promoters. *Mol Cell* 10, 1107-1117.
- Milne, T.A., Hughes, C.M., Lloyd, R., Yang, Z., Rozenblatt-Rosen, O., Dou, Y., Schnepf, R.W., Krankel, C., Livolsi, V.A., Gibbs, D., *et al.* (2005). Menin and MLL cooperatively regulate expression of cyclin-dependent kinase inhibitors. *Proc Natl Acad Sci U S A* 102, 749-754.
- Minowada, J., Onuma, T., and Moore, G.E. (1972). Rosette-forming human lymphoid cell lines. I. Establishment and evidence for origin of thymus-derived lymphocytes. *J Natl Cancer Inst* 49, 891-895.
- Mitelman, F., and Heim, S. (1992). Quantitative acute leukemia cytogenetics. *Genes Chromosomes Cancer* 5, 57-66.
- Mitterbauer-Hohendanner, G., and Mannhalter, C. (2004). The biological and clinical significance of MLL abnormalities in haematological malignancies. *Eur J Clin Invest* 34 Suppl 2, 12-24.
- Morrissey, J., Tkachuk, D.C., Milatovich, A., Francke, U., Link, M., and Cleary, M.L. (1993). A serine/proline-rich protein is fused to HRX in t(4;11) acute leukemias. *Blood* 81, 1124-1131.
- Mueller, D., Bach, C., Zeisig, D., Garcia-Cuellar, M.P., Monroe, S., Sreekumar, A., Zhou, R., Nesvizhskii, A., Chinnaiyan, A., Hess, J.L., *et al.* (2007). A role for the MLL fusion partner ENL in transcriptional elongation and chromatin modification. *Blood* 110, 4445-4454.

- Mueller, D., Garcia-Cuellar, M.P., Bach, C., Buhl, S., Maethner, E., and Slany, R.K. (2009). Misguided transcriptional elongation causes mixed lineage leukemia. *PLoS Biol* 7, e1000249.
- Nakamura, T., Alder, H., Gu, Y., Prasad, R., Canaani, O., Kamada, N., Gale, R.P., Lange, B., Crist, W.M., Nowell, P.C., *et al.* (1993). Genes on chromosomes 4, 9, and 19 involved in 11q23 abnormalities in acute leukemia share sequence homology and/or common motifs. *Proc Natl Acad Sci U S A* 90, 4631-4635.
- Nakamura, T., Mori, T., Tada, S., Krajewski, W., Rozovskaia, T., Wassell, R., Dubois, G., Mazo, A., Croce, C.M., and Canaani, E. (2002). ALL-1 is a histone methyltransferase that assembles a supercomplex of proteins involved in transcriptional regulation. *Mol Cell* 10, 1119-1128.
- Nakanishi, H., Nakamura, T., Canaani, E., and Croce, C.M. (2007). ALL1 fusion proteins induce deregulation of EphA7 and ERK phosphorylation in human acute leukemias. *Proc Natl Acad Sci U S A* 104, 14442-14447.
- Ng, H.H., Robert, F., Young, R.A., and Struhl, K. (2003). Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. *Mol Cell* 11, 709-719.
- Nie, Z., Yan, Z., Chen, E.H., Sechi, S., Ling, C., Zhou, S., Xue, Y., Yang, D., Murray, D., Kanakubo, E., *et al.* (2003). Novel SWI/SNF chromatin-remodeling complexes contain a mixed-lineage leukemia chromosomal translocation partner. *Mol Cell Biol* 23, 2942-2952.
- Nilson, I., Reichel, M., Ennas, M.G., Greim, R., Knorr, C., Siegler, G., Greil, J., Fey, G.H., and Marschalek, R. (1997). Exon/intron structure of the human AF-4 gene, a member of the AF-4/LAF-4/FMR-2 gene family coding for a nuclear protein with structural alterations in acute leukaemia. *Br J Haematol* 98, 157-169.
- Noma, K., and Grewal, S.I. (2002). Histone H3 lysine 4 methylation is mediated by Set1 and promotes maintenance of active chromatin states in fission yeast. *Proc Natl Acad Sci U S A* 99 *Suppl* 4, 16438-16445.
- Oguchi, K., Takagi, M., Tsuchida, R., Taya, Y., Ito, E., Isoyama, K., Ishii, E., Zannini, L., Delia, D., and Mizutani, S. (2003). Missense mutation and defective function of ATM in a childhood acute leukemia patient with MLL gene rearrangement. *Blood* 101, 3622-3627.
- Ohkura, N., Kishi, M., Tsukada, T., and Yamaguchi, K. (2001). Menin, a gene product responsible for multiple endocrine neoplasia type 1, interacts with the putative tumor metastasis suppressor nm23. *Biochem Biophys Res Commun* 282, 1206-1210.
- Okada, Y., Feng, Q., Lin, Y., Jiang, Q., Li, Y., Coffield, V.M., Su, L., Xu, G., and Zhang, Y. (2005). hDOT1L links histone methylation to leukemogenesis. *Cell* 121, 167-178.
- Okada, Y., Jiang, Q., Lemieux, M., Jeannotte, L., Su, L., and Zhang, Y. (2006). Leukaemic transformation by CALM-AF10 involves upregulation of Hoxa5 by hDOT1L. *Nat Cell Biol* 8, 1017-1024.
- Oliver, P.L., Bitoun, E., Clark, J., Jones, E.L., and Davies, K.E. (2004). Mediation of Af4 protein function in the cerebellum by Siah proteins. *Proc Natl Acad Sci U S A* 101, 14901-14906.
- Palermo, C.M., Bennett, C.A., Winters, A.C., and Hemenway, C.S. (2008). The AF4-mimetic peptide, PFWT, induces necrotic cell death in MV4-11 leukemia cells. *Leuk Res* 32, 633-642.

- Patel, A., Dharmarajan, V., and Cosgrove, M.S. (2008a). Structure of WDR5 bound to mixed lineage leukemia protein-1 peptide. *J Biol Chem* 283, 32158-32161.
- Patel, A., Dharmarajan, V., Vought, V.E., and Cosgrove, M.S. (2009). On the mechanism of multiple lysine methylation by the human mixed lineage leukemia protein-1 (MLL1) core complex. *J Biol Chem* 284, 24242-24256.
- Patel, A., Vought, V.E., Dharmarajan, V., and Cosgrove, M.S. (2008b). A conserved arginine-containing motif crucial for the assembly and enzymatic activity of the mixed lineage leukemia protein-1 core complex. *J Biol Chem* 283, 32162-32175.
- Patel, A., Vought, V.E., Dharmarajan, V., and Cosgrove, M.S. (2011). A novel non-SET domain multi-subunit methyltransferase required for sequential nucleosomal histone H3 methylation by the mixed lineage leukemia protein-1 (MLL1) core complex. *J Biol Chem* 286, 3359-3369.
- Poisson, A., Zablewska, B., and Gaudray, P. (2003). Menin interacting proteins as clues toward the understanding of multiple endocrine neoplasia type 1. *Cancer Lett* 189, 1-10.
- Pokholok, D.K., Harbison, C.T., Levine, S., Cole, M., Hannett, N.M., Lee, T.I., Bell, G.W., Walker, K., Rolfe, P.A., Herbolsheimer, E., *et al.* (2005). Genome-wide map of nucleosome acetylation and methylation in yeast. *Cell* 122, 517-527.
- Poppe, B., Vandesompele, J., Schoch, C., Lindvall, C., Mrozek, K., Bloomfield, C.D., Beverloo, H.B., Michaux, L., Dastugue, N., Herens, C., *et al.* (2004). Expression analyses identify MLL as a prominent target of 11q23 amplification and support an etiologic role for MLL gain of function in myeloid malignancies. *Blood* 103, 229-235.
- Prasad, R., Yano, T., Sorio, C., Nakamura, T., Rallapalli, R., Gu, Y., Leshkowitz, D., Croce, C.M., and Canaani, E. (1995). Domains with transcriptional regulatory activity within the ALL1 and AF4 proteins involved in acute leukemia. *Proc Natl Acad Sci U S A* 92, 12160-12164.
- Pray-Grant, M.G., Daniel, J.A., Schieltz, D., Yates, J.R., 3rd, and Grant, P.A. (2005). Chd1 chromodomain links histone H3 methylation with SAGA- and SLIK-dependent acetylation. *Nature* 433, 434-438.
- Pui, C.H., Chessells, J.M., Camitta, B., Baruchel, A., Biondi, A., Boyett, J.M., Carroll, A., Eden, O.B., Evans, W.E., Gardner, H., *et al.* (2003). Clinical heterogeneity in childhood acute lymphoblastic leukemia with 11q23 rearrangements. *Leukemia* 17, 700-706.
- Qian, C., and Zhou, M.M. (2006). SET domain protein lysine methyltransferases: Structure, specificity and catalysis. *Cell Mol Life Sci* 63, 2755-2763.
- Quentmeier, H., Reinhardt, J., Zaborski, M., and Drexler, H.G. (2003). MLL partial tandem duplications in acute leukemia cell lines. *Leukemia* 17, 980-981.
- Ross, M.E., Mahfouz, R., Onciu, M., Liu, H.C., Zhou, X., Song, G., Shurtleff, S.A., Pounds, S., Cheng, C., Ma, J., *et al.* (2004). Gene expression profiling of pediatric acute myelogenous leukemia. *Blood* 104, 3679-3687.
- Roudaia, L., and Speck, N.A. (2008). A MENage a Trois in leukemia. *Cancer Cell* 14, 3-5.
- Rozenblatt-Rosen, O., Rozovskaia, T., Burakov, D., Sedkov, Y., Tillib, S., Blechman, J., Nakamura, T., Croce, C.M., Mazo, A., and Canaani, E. (1998). The C-terminal SET domains of ALL-1 and TRITHORAX interact with the INI1 and SNR1 proteins, components of the SWI/SNF complex. *Proc Natl Acad Sci U S A* 95, 4152-4157.

- Rozovskaia, T., Feinstein, E., Mor, O., Foa, R., Blechman, J., Nakamura, T., Croce, C.M., Cimino, G., and Canaani, E. (2001). Upregulation of Meis1 and HoxA9 in acute lymphocytic leukemias with the t(4 : 11) abnormality. *Oncogene* 20, 874-878.
- Rubnitz, J.E., Link, M.P., Shuster, J.J., Carroll, A.J., Hakami, N., Frankel, L.S., Pullen, D.J., and Cleary, M.L. (1994a). Frequency and prognostic significance of HRX rearrangements in infant acute lymphoblastic leukemia: a Pediatric Oncology Group study. *Blood* 84, 570-573.
- Rubnitz, J.E., Morrissey, J., Savage, P.A., and Cleary, M.L. (1994b). ENL, the gene fused with HRX in t(11;19) leukemias, encodes a nuclear protein with transcriptional activation potential in lymphoid and myeloid cells. *Blood* 84, 1747-1752.
- Ruthenburg, A.J., Wang, W., Graybosch, D.M., Li, H., Allis, C.D., Patel, D.J., and Verdine, G.L. (2006). Histone H3 recognition and presentation by the WDR5 module of the MLL1 complex. *Nat Struct Mol Biol* 13, 704-712.
- Santos-Rosa, H., Schneider, R., Bannister, A.J., Sherriff, J., Bernstein, B.E., Emre, N.C., Schreiber, S.L., Mellor, J., and Kouzarides, T. (2002). Active genes are tri-methylated at K4 of histone H3. *Nature* 419, 407-411.
- Santos-Rosa, H., Schneider, R., Bernstein, B.E., Karabetsou, N., Morillon, A., Weise, C., Schreiber, S.L., Mellor, J., and Kouzarides, T. (2003). Methylation of histone H3 K4 mediates association of the Isw1p ATPase with chromatin. *Mol Cell* 12, 1325-1332.
- Scharf, S., Zech, J., Bursen, A., Schraets, D., Oliver, P.L., Kliem, S., Pfitzner, E., Gillert, E., Dingermann, T., and Marschalek, R. (2007). Transcription linked to recombination: a gene-internal promoter coincides with the recombination hot spot II of the human MLL gene. *Oncogene* 26, 1361-1371.
- Schichman, S.A., Caligiuri, M.A., Strout, M.P., Carter, S.L., Gu, Y., Canaani, E., Bloomfield, C.D., and Croce, C.M. (1994). ALL-1 tandem duplication in acute myeloid leukemia with a normal karyotype involves homologous recombination between Alu elements. *Cancer Res* 54, 4277-4280.
- Schichman, S.A., Canaani, E., and Croce, C.M. (1995). Self-fusion of the ALL1 gene. A new genetic mechanism for acute leukemia. *Jama* 273, 571-576.
- Schneider, R., Bannister, A.J., Myers, F.A., Thorne, A.W., Crane-Robinson, C., and Kouzarides, T. (2004). Histone H3 lysine 4 methylation patterns in higher eukaryotic genes. *Nat Cell Biol* 6, 73-77.
- Schnepp, R.W., Hou, Z., Wang, H., Petersen, C., Silva, A., Masai, H., and Hua, X. (2004). Functional interaction between tumor suppressor menin and activator of S-phase kinase. *Cancer Res* 64, 6791-6796.
- Schoch, C., Schnittger, S., Klaus, M., Kern, W., Hiddemann, W., and Haferlach, T. (2003). AML with 11q23/MLL abnormalities as defined by the WHO classification: incidence, partner chromosomes, FAB subtype, age distribution, and prognostic impact in an unselected series of 1897 cytogenetically analyzed AML cases. *Blood* 102, 2395-2402.
- Schreiner, S.A., Garcia-Cuellar, M.P., Fey, G.H., and Slany, R.K. (1999). The leukemogenic fusion of MLL with ENL creates a novel transcriptional transactivator. *Leukemia* 13, 1525-1533.
- Schubeler, D., MacAlpine, D.M., Scalzo, D., Wirbelauer, C., Kooperberg, C., van Leeuwen, F., Gottschling, D.E., O'Neill, L.P., Turner, B.M., Delrow, J., *et al.* (2004). The histone

- modification pattern of active genes revealed through genome-wide chromatin analysis of a higher eukaryote. *Genes Dev* 18, 1263-1271.
- Schuetz, A., Allali-Hassani, A., Martin, F., Loppnau, P., Vedadi, M., Bochkarev, A., Plotnikov, A.N., Arrowsmith, C.H., and Min, J. (2006). Structural basis for molecular recognition and presentation of histone H3 by WDR5. *Embo J* 25, 4245-4252.
- Shapiro, G.I. (2006). Cyclin-dependent kinase pathways as targets for cancer treatment. *J Clin Oncol* 24, 1770-1783.
- Simone, F., Polak, P.E., Kaberlein, J.J., Luo, R.T., Levitan, D.A., and Thirman, M.J. (2001). EAF1, a novel ELL-associated factor that is delocalized by expression of the MLL-ELL fusion protein. *Blood* 98, 201-209.
- Sims, R.J., 3rd, Chen, C.F., Santos-Rosa, H., Kouzarides, T., Patel, S.S., and Reinberg, D. (2005). Human but not yeast CHD1 binds directly and selectively to histone H3 methylated at lysine 4 via its tandem chromodomains. *J Biol Chem* 280, 41789-41792.
- Slany, R.K. (2009). The molecular biology of mixed lineage leukemia. *Haematologica* 94, 984-993.
- So, C.W., Karsunky, H., Passegue, E., Cozzio, A., Weissman, I.L., and Cleary, M.L. (2003). MLL-GAS7 transforms multipotent hematopoietic progenitors and induces mixed lineage leukemias in mice. *Cancer Cell* 3, 161-171.
- So, C.W., Karsunky, H., Wong, P., Weissman, I.L., and Cleary, M.L. (2004). Leukemic transformation of hematopoietic progenitors by MLL-GAS7 in the absence of Hoxa7 or Hoxa9. *Blood* 103, 3192-3199.
- Sobulo, O.M., Borrow, J., Tomek, R., Reshmi, S., Harden, A., Schlegelberger, B., Housman, D., Doggett, N.A., Rowley, J.D., and Zeleznik-Le, N.J. (1997). MLL is fused to CBP, a histone acetyltransferase, in therapy-related acute myeloid leukemia with a t(11;16)(q23;p13.3). *Proc Natl Acad Sci U S A* 94, 8732-8737.
- Song, J.J., and Kingston, R.E. (2008). WDR5 interacts with mixed lineage leukemia (MLL) protein via the histone H3-binding pocket. *J Biol Chem* 283, 35258-35264.
- Southall, S.M., Wong, P.S., Odho, Z., Roe, S.M., and Wilson, J.R. (2009). Structural basis for the requirement of additional factors for MLL1 SET domain activity and recognition of epigenetic marks. *Mol Cell* 33, 181-191.
- Sowa, H., Kaji, H., Hendy, G.N., Canaff, L., Komori, T., Sugimoto, T., and Chihara, K. (2004). Menin is required for bone morphogenetic protein 2- and transforming growth factor beta-regulated osteoblastic differentiation through interaction with Smads and Runx2. *J Biol Chem* 279, 40267-40275.
- Srinivasan, R.S., de Erkenez, A.C., and Hemenway, C.S. (2003). The mixed lineage leukemia fusion partner AF9 binds specific isoforms of the BCL-6 corepressor. *Oncogene* 22, 3395-3406.
- Srinivasan, R.S., Nesbit, J.B., Marrero, L., Erfurth, F., LaRussa, V.F., and Hemenway, C.S. (2004). The synthetic peptide PFWT disrupts AF4-AF9 protein complexes and induces apoptosis in t(4;11) leukemia cells. *Leukemia* 18, 1364-1372.
- Stam, R.W., den Boer, M.L., Passier, M.M., Janka-Schaub, G.E., Sallan, S.E., Armstrong, S.A., and Pieters, R. (2006). Silencing of the tumor suppressor gene FHIT is highly characteristic for MLL gene rearranged infant acute lymphoblastic leukemia. *Leukemia* 20, 264-271.

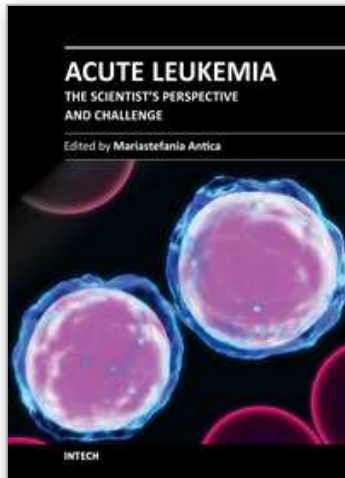
- Steward, M.M., Lee, J.S., O'Donovan, A., Wyatt, M., Bernstein, B.E., and Shilatifard, A. (2006). Molecular regulation of H3K4 trimethylation by ASH2L, a shared subunit of MLL complexes. *Nat Struct Mol Biol* 13, 852-854.
- Stewart, C., Parente, F., Piehl, F., Farnebo, F., Quincey, D., Silins, G., Bergman, L., Carle, G.F., Lemmens, I., Grimmond, S., *et al.* (1998). Characterization of the mouse Men1 gene and its expression during development. *Oncogene* 17, 2485-2493.
- Stoller, J.Z., Huang, L., Tan, C.C., Huang, F., Zhou, D.D., Yang, J., Gelb, B.D., and Epstein, J.A. Ash2l interacts with Tbx1 and is required during early embryogenesis. *Exp Biol Med (Maywood)* 235, 569-576.
- Stong, R.C., Korsmeyer, S.J., Parkin, J.L., Arthur, D.C., and Kersey, J.H. (1985). Human acute leukemia cell line with the t(4;11) chromosomal rearrangement exhibits B lineage and monocytic characteristics. *Blood* 65, 21-31.
- Strahl, B.D., Ohba, R., Cook, R.G., and Allis, C.D. (1999). Methylation of histone H3 at lysine 4 is highly conserved and correlates with transcriptionally active nuclei in Tetrahymena. *Proc Natl Acad Sci U S A* 96, 14967-14972.
- Streubel, B., Valent, P., Jager, U., Edelhauser, M., Wandt, H., Wagner, T., Buchner, T., Lechner, K., and Fonatsch, C. (2000). Amplification of the MLL gene on double minutes, a homogeneously staining region, and ring chromosomes in five patients with acute myeloid leukemia or myelodysplastic syndrome. *Genes Chromosomes Cancer* 27, 380-386.
- Stubbs, M.C., Kim, Y.M., Krivtsov, A.V., Wright, R.D., Feng, Z., Agarwal, J., Kung, A.L., and Armstrong, S.A. (2008). MLL-AF9 and FLT3 cooperation in acute myelogenous leukemia: development of a model for rapid therapeutic assessment. *Leukemia* 22, 66-77.
- Su, M.A., Wisotzkey, R.G., and Newfeld, S.J. (2001). A screen for modifiers of decapentaplegic mutant phenotypes identifies lilliputian, the only member of the Fragile-X/Burkitt's Lymphoma family of transcription factors in *Drosophila melanogaster*. *Genetics* 157, 717-725.
- Sukhodolets, K.E., Hickman, A.B., Agarwal, S.K., Sukhodolets, M.V., Obungu, V.H., Novotny, E.A., Crabtree, J.S., Chandrasekharappa, S.C., Collins, F.S., Spiegel, A.M., *et al.* (2003). The 32-kilodalton subunit of replication protein A interacts with menin, the product of the MEN1 tumor suppressor gene. *Mol Cell Biol* 23, 493-509.
- Takahashi, Y.H., Lee, J.S., Swanson, S.K., Saraf, A., Florens, L., Washburn, M.P., Trievel, R.C., and Shilatifard, A. (2009). Regulation of H3K4 trimethylation via Cps40 (Spp1) of COMPASS is monoubiquitination independent: implication for a Phe/Tyr switch by the catalytic domain of Set1. *Mol Cell Biol* 29, 3478-3486.
- Takeda, S., Chen, D.Y., Westergard, T.D., Fisher, J.K., Rubens, J.A., Sasagawa, S., Kan, J.T., Korsmeyer, S.J., Cheng, E.H., and Hsieh, J.J. (2006). Proteolysis of MLL family proteins is essential for caspase-1-orchestrated cell cycle progression. *Genes Dev* 20, 2397-2409.
- Taketani, T., Taki, T., Sugita, K., Furuichi, Y., Ishii, E., Hanada, R., Tsuchida, M., Sugita, K., Ida, K., and Hayashi, Y. (2004). FLT3 mutations in the activation loop of tyrosine kinase domain are frequently found in infant ALL with MLL rearrangements and pediatric ALL with hyperdiploidy. *Blood* 103, 1085-1088.

- Taki, T., Kano, H., Taniwaki, M., Sako, M., Yanagisawa, M., and Hayashi, Y. (1999). AF5q31, a newly identified AF4-related gene, is fused to MLL in infant acute lymphoblastic leukemia with ins(5;11)(q31;q13q23). *Proc Natl Acad Sci U S A* 96, 14535-14540.
- Taki, T., Sako, M., Tsuchida, M., and Hayashi, Y. (1997). The t(11;16)(q23;p13) translocation in myelodysplastic syndrome fuses the MLL gene to the CBP gene. *Blood* 89, 3945-3950.
- Tan, C.C., Sindhu, K.V., Li, S., Nishio, H., Stoller, J.Z., Oishi, K., Puttreddy, S., Lee, T.J., Epstein, J.A., Walsh, M.J., *et al.* (2008). Transcription factor Ap2delta associates with Ash2l and ALR, a trithorax family histone methyltransferase, to activate Hoxc8 transcription. *Proc Natl Acad Sci U S A* 105, 7472-7477.
- Terranova, R., Agherbi, H., Boned, A., Meresse, S., and Djabali, M. (2006). Histone and DNA methylation defects at Hox genes in mice expressing a SET domain-truncated form of Mll. *Proc Natl Acad Sci U S A* 103, 6629-6634.
- Thakker, R.V. (2001). Multiple endocrine neoplasia. *Horm Res* 56 Suppl 1, 67-72.
- Tkachuk, D.C., Kohler, S., and Cleary, M.L. (1992). Involvement of a homolog of Drosophila trithorax by 11q23 chromosomal translocations in acute leukemias. *Cell* 71, 691-700.
- Trievel, R.C., and Shilatifard, A. (2009). WDR5, a complexed protein. *Nat Struct Mol Biol* 16, 678-680.
- Tsuchiya, S., Yamabe, M., Yamaguchi, Y., Kobayashi, Y., Konno, T., and Tada, K. (1980). Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). *Int J Cancer* 26, 171-176.
- Tyagi, S., Chabes, A.L., Wysocka, J., and Herr, W. (2007). E2F activation of S phase promoters via association with HCF-1 and the MLL family of histone H3K4 methyltransferases. *Mol Cell* 27, 107-119.
- von Bergh, A., Gargallo, P., De Prijck, B., Vranckx, H., Marschalek, R., Larripa, I., Kluin, P., Schuurung, E., and Hagemeijer, A. (2001). Cryptic t(4;11) encoding MLL-AF4 due to insertion of 5' MLL sequences in chromosome 4. *Leukemia* 15, 595-600.
- von Bergh, A.R., Beverloo, H.B., Rombout, P., van Wering, E.R., van Weel, M.H., Beverstock, G.C., Kluin, P.M., Slater, R.M., and Schuurung, E. (2002). LAF4, an AF4-related gene, is fused to MLL in infant acute lymphoblastic leukemia. *Genes Chromosomes Cancer* 35, 92-96.
- Wang, J., Iwasaki, H., Krivtsov, A., Febbo, P.G., Thorner, A.R., Ernst, P., Anastasiadou, E., Kutok, J.L., Kogan, S.C., Zinkel, S.S., *et al.* (2005). Conditional MLL-CBP targets GMP and models therapy-related myeloproliferative disease. *Embo J* 24, 368-381.
- Wang, Z., Smith, K.S., Murphy, M., Piloto, O., Somerville, T.C., and Cleary, M.L. (2008). Glycogen synthase kinase 3 in MLL leukaemia maintenance and targeted therapy. *Nature* 455, 1205-1209.
- Welch, M.D., and Drubin, D.G. (1994). A nuclear protein with sequence similarity to proteins implicated in human acute leukemias is important for cellular morphogenesis and actin cytoskeletal function in *Saccharomyces cerevisiae*. *Mol Biol Cell* 5, 617-632.
- Whitman, S.P., Hackanson, B., Liyanarachchi, S., Liu, S., Rush, L.J., Maharry, K., Margeson, D., Davuluri, R., Wen, J., Witte, T., *et al.* (2008). DNA hypermethylation and epigenetic silencing of the tumor suppressor gene, SLC5A8, in acute myeloid leukemia with the MLL partial tandem duplication. *Blood* 112, 2013-2016.

- Whitman, S.P., Liu, S., Vukosavljevic, T., Rush, L.J., Yu, L., Liu, C., Klisovic, M.I., Maharry, K., Guimond, M., Strout, M.P., *et al.* (2005). The MLL partial tandem duplication: evidence for recessive gain-of-function in acute myeloid leukemia identifies a novel patient subgroup for molecular-targeted therapy. *Blood* 106, 345-352.
- Wiederschain, D., Kawai, H., Shilatifard, A., and Yuan, Z.M. (2005). Multiple mixed lineage leukemia (MLL) fusion proteins suppress p53-mediated response to DNA damage. *J Biol Chem* 280, 24315-24321.
- Wu, X., and Hua, X. (2008). Menin, histone h3 methyltransferases, and regulation of cell proliferation: current knowledge and perspective. *Curr Mol Med* 8, 805-815.
- Wysocka, J., Swigut, T., Milne, T.A., Dou, Y., Zhang, X., Burlingame, A.L., Roeder, R.G., Brivanlou, A.H., and Allis, C.D. (2005). WDR5 associates with histone H3 methylated at K4 and is essential for H3 K4 methylation and vertebrate development. *Cell* 121, 859-872.
- Wysocka, J., Swigut, T., Xiao, H., Milne, T.A., Kwon, S.Y., Landry, J., Kauer, M., Tackett, A.J., Chait, B.T., Badenhorst, P., *et al.* (2006). A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. *Nature* 442, 86-90.
- Xia, Z.B., Anderson, M., Diaz, M.O., and Zeleznik-Le, N.J. (2003). MLL repression domain interacts with histone deacetylases, the polycomb group proteins HPC2 and BMI-1, and the corepressor C-terminal-binding protein. *Proc Natl Acad Sci U S A* 100, 8342-8347.
- Yagi, H., Deguchi, K., Aono, A., Tani, Y., Kishimoto, T., and Komori, T. (1998). Growth disturbance in fetal liver hematopoiesis of Mll-mutant mice. *Blood* 92, 108-117.
- Yamashita, M., Hirahara, K., Shinnakasu, R., Hosokawa, H., Norikane, S., Kimura, M.Y., Hasegawa, A., and Nakayama, T. (2006). Crucial role of MLL for the maintenance of memory T helper type 2 cell responses. *Immunity* 24, 611-622.
- Yao, Q., Nishiuchi, R., Kitamura, T., and Kersey, J.H. (2005). Human leukemias with mutated FLT3 kinase are synergistically sensitive to FLT3 and Hsp90 inhibitors: the key role of the STAT5 signal transduction pathway. *Leukemia* 19, 1605-1612.
- Yasui, H., Hideshima, T., Hamasaki, M., Roccaro, A.M., Shiraishi, N., Kumar, S., Tassone, P., Ishitsuka, K., Raje, N., Tai, Y.T., *et al.* (2005). SDX-101, the R-enantiomer of etodolac, induces cytotoxicity, overcomes drug resistance, and enhances the activity of dexamethasone in multiple myeloma. *Blood* 106, 706-712.
- Yeoh, E.J., Ross, M.E., Shurtleff, S.A., Williams, W.K., Patel, D., Mahfouz, R., Behm, F.G., Raimondi, S.C., Relling, M.V., Patel, A., *et al.* (2002). Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. *Cancer Cell* 1, 133-143.
- Yocum, A.K., Busch, C.M., Felix, C.A., and Blair, I.A. (2006). Proteomics-based strategy to identify biomarkers and pharmacological targets in leukemias with t(4;11) translocations. *J Proteome Res* 5, 2743-2753.
- Yokoyama, A., and Cleary, M.L. (2008). Menin critically links MLL proteins with LEDGF on cancer-associated target genes. *Cancer Cell* 14, 36-46.
- Yokoyama, A., Kitabayashi, I., Ayton, P.M., Cleary, M.L., and Ohki, M. (2002). Leukemia proto-oncoprotein MLL is proteolytically processed into 2 fragments with opposite transcriptional properties. *Blood* 100, 3710-3718.

- Yokoyama, A., Somervaille, T.C., Smith, K.S., Rozenblatt-Rosen, O., Meyerson, M., and Cleary, M.L. (2005). The menin tumor suppressor protein is an essential oncogenic cofactor for MLL-associated leukemogenesis. *Cell* 123, 207-218.
- Yokoyama, A., Wang, Z., Wysocka, J., Sanyal, M., Aufiero, D.J., Kitabayashi, I., Herr, W., and Cleary, M.L. (2004). Leukemia proto-oncoprotein MLL forms a SET1-like histone methyltransferase complex with menin to regulate Hox gene expression. *Mol Cell Biol* 24, 5639-5649.
- Yu, B.D., Hess, J.L., Horning, S.E., Brown, G.A., and Korsmeyer, S.J. (1995). Altered Hox expression and segmental identity in Mll-mutant mice. *Nature* 378, 505-508.
- Zeisig, B.B., Milne, T., Garcia-Cuellar, M.P., Schreiner, S., Martin, M.E., Fuchs, U., Borkhardt, A., Chanda, S.K., Walker, J., Soden, R., *et al.* (2004). Hoxa9 and Meis1 are key targets for MLL-ENL-mediated cellular immortalization. *Mol Cell Biol* 24, 617-628.
- Zhu, E.D., Demay, M.B., and Gori, F. (2008). Wdr5 is essential for osteoblast differentiation. *J Biol Chem* 283, 7361-7367.
- Ziemin-van der Poel, S., McCabe, N.R., Gill, H.J., Espinosa, R., 3rd, Patel, Y., Harden, A., Rubinelli, P., Smith, S.D., LeBeau, M.M., Rowley, J.D., *et al.* (1991). Identification of a gene, MLL, that spans the breakpoint in 11q23 translocations associated with human leukemias. *Proc Natl Acad Sci U S A* 88, 10735-10739.

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Acute Leukemia - The Scientist's Perspective and Challenge

Edited by Prof. Mariastefania Antica

ISBN 978-953-307-553-2

Hard cover, 428 pages

Publisher InTech

Published online 22, December, 2011

Published in print edition December, 2011

This book provides a comprehensive overview of the basic mechanisms underlying areas of acute leukemia, current advances, and future directions in management of this disease. The first section discusses the classification of acute leukemia, taking into account diagnoses dependent on techniques that are essential, and thankfully readily available, in the laboratory. The second section concerns recent advances in molecular biology, markers, receptors, and signaling molecules responsible for disease progression, diagnostics based on biochips and other molecular genetic analysis. These advances provide clinicians with important understanding and improved decision making towards the most suitable therapy for acute leukemia. Biochemical, structural, and genetic studies may bring a new era of epigenetic based drugs along with additional molecular targets that will form the basis for novel treatment strategies. Later in the book, pediatric acute leukemia is covered, emphasizing that children are not small adults when it comes to drug development. The last section is a collection of chapters about treatment, as chemotherapy-induced toxicity is still a significant clinical concern. The present challenge lies in reducing the frequency and seriousness of adverse effects while maintaining efficacy and avoiding over-treatment of patients.

How to reference

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Venkatasubramanian Dharmarajan and Michael S. Cosgrove (2011). Biochemistry of the Mixed Lineage Leukemia 1 (MLL1) Protein and Targeted Therapies for Associated Leukemia, Acute Leukemia - The Scientist's Perspective and Challenge, Prof. Mariastefania Antica (Ed.), ISBN: 978-953-307-553-2, InTech, Available from: <http://www.intechopen.com/books/acute-leukemia-the-scientist-s-perspective-and-challenge/biochemistry-of-the-mixed-lineage-leukemia-1-ml1-protein-and-targeted-therapies-for-associated-leuk>

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