# A role for DOT1L in *MLL*-rearranged leukemias

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Leukemias harboring rearrangements of the *MLL* gene carry a poor prognosis. Over the past 6 years, it has become increasingly clear that fusions of *MLL* induce widespread epigenetic dysregulation that may mediate much of their transforming activity. The histone methyltransferase DOT1L, which methylates histone 3 on lysine 79 (H3K79) has received particular attention. Several *MLL* fusions may physically interact with DOT1L. Genome-wide H3K79 methylation profiles in *MLL*-rearranged leukemias are abnormal, and can serve to distinguish *MLL*-rearranged from other types of leukemias. Loss of H3K79 methylation affects expression of *MLL*-target loci and is detrimental to the leukemogenic activity of *MLL*-rearranged cells, suggesting that transformation in these leukemias is driven by a DOT1L dependent, aberrant epigenetic program.

The 'histone code hypothesis' proposes that histone modifications, along with proteins that recognize, place and remove these marks, form a sophisticated regulatory network that directly control gene expression. Histone modifications may prime genes for rapid induction after signaling receptor engagement, coordinate individual genes to genetic programs that are coregulated, or organize a sequence of transcriptional events during development. *MLL*-rearranged leukemias have recently been proposed to rely heavily on epigenetic dysregulation during malignant transformation. In contrast to most other cancers, *MLL*-rearranged leukemias display a remarkable paucity of DNA sequence alterations: frequently, the only genetic abnormality uncovered by genome-wide technologies in these leukemias is the *MLL*-translocation. The induction of widespread epigenetic changes could explain the apparent lack of a need for cooperating mutagenesis on a DNA-sequence level. This is significant, since most *MLL*-rearranged leukemias have a poor prognosis, and epigenetic alterations may be amenable to targeted pharmacologic modulation as a new therapeutic strategy.

The histone methyltransferase DOT1L is a strong candidate to mediate much of the epigenetic dysregulation observed in *MLL*-rearranged leukemias. DOT1L methylates H3K79. It is the only known histone methyltransferase for this residue and catalyzes mono-, di- and tri-methylation. H3K79 methylation in eukaryotes is predominantly associated with actively transcribed loci [1]. By association, it has been proposed that H3K79 methylation directly regulates gene expression, and by extension the leukemic transformation of *MLL*-rearranged leukemias. However, the exact molecular mechanisms for this presumed pathway are not known, and H3K79 methyation has also been proposed to reflect transcriptional activity rather than directly control gene expression. In addition, the histone code hypothesis itself remains a matter of scientific debate.

## Physical interaction between DOT1L & MLL fusions

MLL–AF10 was the first *MLL* fusion that has been shown to physically interact with DOT1L [2]. Subsequently, Bitoun *et al.* [3] and Mueller *et al.* [4,5] reported that the common *MLL*-fusion partners AF4, AF5, AF9, AF10 and ENL exist in a complex that also contained DOT1L and pTEFb (a complex of cyclinT and CDK9, which phosphorylates stalled RNA polymerase II [Pol II] and directly stimulates elongation). This complex was termed ENL-associated protein (EAP). Its discovery prompted the hypothesis that each member of the EAP complex, when fused to *MLL*, miss recruits the other EAPs including DOT1L and pTEFb to *MLL* target loci. DOT1L would then presumably methylate H3K79 to induce an

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open chromatin formation, and pTEFb would stimulate Pol II to transcribe the respective locus. Subsequent studies have modified this hypothesis. Multiple groups described similar complexes, termed super elongation complex (SEC), containing AF4, AF9, ENL, AFF4, ELL1 and pTEFb [6], AEP (containing AF4, AF5, ENL and pTEFb) [6,7], and DotCom (containing DOT1L, AF9, ENL, AF10, AF17 along with several WNT pathway modifiers) [8]. DOT1L and pTEFb appear to be the 'effector units' of these complexes. However, none of these complexes contained both DOT1L and pTEFb. In addition, binding site mapping of the interactions between DOT1L, pTEFb and other members of EAP/SEC/AEP/DotCom revealed that many interactions utilize the same domains and are therefore mutually exclusive. For example, ENL and DOT1L are able to bind either pTEFb or AF4, but not both at the same time. pTEFb can bind either DOT1L or AF4. In addition, the above mentioned complexes are not the only modifiers of chromatin and Pol II function recruited by *MLL*-fusions. The $NH_3$-terminal portion of both full length *MLL* and *MLL*-fusion has been shown to recruit the PAFc complex, which associates with initiating and elongating Pol II, and mediates ubiquitination of histone H2B lysine 120 (H2B-K120) by Rad6/Bre1 (human BRE1A/RNF20 and BRE1B/RNF40) [9]. H2B-K120 ubiquitination in turn stimulates H3K79 trimethylation. The exact molecular interaction between *MLL*-fusions, Pol II, pTEFb, DOT1L/H3K79 methylation and other chromatin modifications are thus not fully understood.

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The divergent results obtained when defining the composition of the various complexes are likely due to technical as well as biological differences. The best current evidence points towards the existence of several complexes that are highly context dependent, with the exact composition of DOT1L containing complexes perhaps being influenced by the fusion partner [10]. Both pTEFb and DOT1L appear to be recruited to MLL-fusion target loci either together or sequentially, and to be required for leukemogenesis. Whether H3K79 methylation precedes pTEFb recruitment, or whether it follows release of stalled Pol II and transcriptional elongation remains unresolved.

## H3K79 methylation in *MLL* fusion-driven leukemia: results from genome-wide chromatin immunoprecipitation studies

Early evidence that H3K79 methylation may play a role in *MLL*-fusion mediated gene- expression control stems from experiments demonstrating high levels of H3K79 methylation on the prominent *MLL*-fusion downstream target loci *HOXA9* and *MEIS1* in MLL-ENL transformed hematopoietic progenitors [11]. Subsequent genome-wide analysis revealed a distinct pattern of H3K79 methylation in a *MLL–AF4* mouse model, and in human *MLL*-rearranged primary leukemia samples compared with normal proB cells and leukemias with other cytogenetic abnormalities [12]. This link was further strengthened by the analysis of H3K79 methylation patterns on MLL–AF4 direct target loci in a human *MLL*-rearranged leukemia cell line, and MLL–AF9 direct targets in a mouse model [13,14]. Most *MLL*-fusion target genes are associated with histones methylated at H3K79, and H3K79 methylation patterns on MLL–AF9 target loci appear abnormal [14]. Physiologically, normal H3K79 methylation patterns are characterized by a sharp peak just downstream of the transcription start site, and a slower decrease over the next 500–1000 bp. By comparison, MLL–AF9 targets displayed a higher peak and wider spread to both sides of the transcription start site. This was specific to MLL targets, as nontargets with similar expression levels displayed normal H3K79 methylation patterns. In addition, H3K79 methylation patterns of MLL–AF9 targets were normal in normal hematopoietic progenitors, where many of the same loci are transcribed under physiological regulation, and in myeloid leukemias with a normal (i.e., non-*MLL*-rearranged) karyotype. The abnormal pattern of H3K79 methylation and strong correlation with *MLL*-fusion target, loci has been termed an 'epigenetic lesion' [13,14] and this suggests that these loci might be particularly dependent on this histone modification to maintain expression.

## DOT1L loss-of-function studies

In the past year, several groups have used pharmacologic inhibition, shRNA-knockdown, or conditional knockout models to confirm that DOT1L is required for full *MLL*-fusion mediated leukemic transformation, and *in vivo* leukemia development and maintenance [14–18]. Fusions that appear to require DOT1L include MLL–AF4 [14,15], MLL–AF9 [14,16–18], and possibly MLL–GAS7 [17]. Results for MLL–AFX are less clear [17,18]. Careful analysis of H3K79

methylation profiles and gene-expression changes after loss of DOT1L in MLL–AF9 driven leukemia cells revealed that despite a large number of loci associated with H3K79, only a small subset of genes demonstrate a change in expression soon after DOT1L inactivation. This set of genes is highly enriched for MLL–AF9 targets and other genes with known functional significance in *MLL*-rearranged leukemia biology [14]. *MLL*-rearranged leukemias thus appear to be driven by an aberrant genetic program that is defined by its association with H3K79 methylated histones, and dependent on this epigenetic modification. Leukemias driven by different leukemogenic fusions such as E2A–HLF [16] and E2A–Pbx1 [17], or by ectopic expression of the major *MLL*-fusion downstream targets HoxA9 and Meis1a from a retroviral promoter [14] were insensitive to loss of DOT1L. *In vitro* and *in vivo* efficacy of an *S*-adenosyl methionine competitive small molecule inhibitor of DOT1L in human *MLL*-rearranged leukemia cell lines confirmed the effects observed with genetic loss of DOT1L can be recapitulated by pharmacologic inhibition of its catalytic activity [15]. At this point, it appears to be established that functional DOT1L is required for the most common *MLL*-rearranged leukemias, and displays specificity with respect to several other types of leukemia. Two important questions remain. First, while H3K79 methylation plays a role, the molecular consequences of the presence of this epigenetic mark are not understood. Potential roles could include inhibition of silencing, facilitation of elongation (by serving as a docking site for elongation associated proteins), or denoting actively transcribed regions through cell division for rapid re-establishment of an active configuration of such marked loci. Second, evaluation of normal hematopoiesis after loss of DOT1L has revealed pancytopenia that ranged from mild/moderate to severe, depending on the experimental system [14,16,18]. It is unclear to which degree this is reversible, and raises some concern for hematologic side effects of pharmacologic inhibition of DOT1L in a clinical setting. However, the effect of complete and irreversible loss of DOT1L may be very different from temporal inhibition of its catalytic activity. An accurate assessment of hematopoietic and other toxicities, and the width of a therapeutic window, has to await clinical development of pharmacologic inhibitors of DOT1L.

So how important is DOT1L in *MLL*-rearranged leukemias? We believe that a crucial role for DOT1L in *MLL*-rearranged leukemias is now well established. This is strongly supported by the functional evidence demonstrating that a loss of DOT1L profoundly affects the leukemogenic gene expression program and leukemogenic activity of *MLL* fusion-transformed cells, while several non-*MLL* leukemia cells are unaffected. What exactly does DOT1L do? This is still very much a matter of debate, and will require further studies. In the mean time, the development of small molecule inhibitors for DOT1L is clearly warranted. Such molecules will help to address some of the outstanding mechanistic questions, and most importantly, they may have a chance to change the outlook for patients with this devastating type of leukemia.

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