
EPIGENETICS

# Histone methylation: it's in the numbers

The expression of homeobox (HOX) proteins, which are important regulators of developmental transcription programmes, is altered in approximately 50% of cases of acute myeloid leukaemia (AML). In particular, the expression of the HOXA cluster is altered in AML with mixed-lineage leukaemia (MLL) rearrangements, and misdirection of DOT1L — a histone methyltransferase that catalyses histone H3 lysine 79 (H3K79) methylation — to target genes of MLL fusion proteins is thought to drive gene hyperexpression.

To examine the role of DOT1L in the regulation of HOXA gene expression, Deshpande *et al.* sorted lineage$^-$ SCA1$^+$ KIT$^+$ (LSK) cells, which are enriched in haematopoietic stem cells (HSCs), from the bone marrow of mice with inducible *Dot1l* deletion. Chromatin immunoprecipitation (ChIP) for H3K79 monomethylation (H3K79me1), H3K79me2 or H3K79me3 in LSKs and granulocyte-macrophage progenitors (GMPs; which have low expression levels of the *Hoxa7–10* cluster and *Meis1*) revealed that H3K79me2 and H3K79me3 levels were reduced at these loci in GMPs compared with LSKs. On examining MLL–AF9 leukaemias, they found that HOXA gene promoters had high levels of H3K79me2 and H3K79me3 and low levels of H3K79me1. This indicates that the hyperexpression of HOXA genes is driven by the conversion of H3K79me1 to H3K79me2 and H3K79me3, and that H3K79me1 is insufficient to sustain high levels of gene expression.

> “DOT1L–AF10 regulates HOXA gene expression”

Next, the authors investigated how H3K79me1 conversion to higher methylation states was regulated. Having excluded a role for changes in DOT1L expression, they investigated whether a cofactor might enhance DOT1L recruitment and activity. They found that AF10 knockdown reduced H3K79me2 levels (but not H3K79me1 levels), showing that AF10 is crucial for the conversion of H3K79me1 to H3K79me2. *Af10* deletion also reduced the proliferation of leukaemia cell lines with MLL fusion proteins and impaired colony formation of MLL–AF9 and MLL–AF6 transformed cells. *Af10* deletion also delayed the initiation and decreased the propagation of leukaemia in secondary recipient mice of MLL–AF9 and MLL–AF6 transformed cells. Moreover, the authors found that *Af10*-deficient MLL–AF9 leukaemia cells showed an increased sensitivity to the DOT1L inhibitor, EPZ004777, indicating that AF10 could be an effective therapeutic target.

ChIP followed by sequencing (ChIP–seq) of *Af10*-deficient leukaemias revealed that locus-specific changes to H3K79 methylation particularly decreased H3K79me2 and H3K79me3 levels at the *Hoxa7–10* cluster, whereas H3K79me1 levels were the same or increased. A genome-wide analysis of H3K79me1 and H3K79me2 levels showed that genes with decreased H3K79me2 and increased H3K79me1 levels were enriched for MLL–AF9 target genes. Indeed, *Af10* deletion affected the ratio of H3K79me1:H3K79me2 at most of the 129 MLL–AF9 target genes and restored H3K79 methylation levels to those observed in cells that do not express MLL–AF9. The expression of most of the MLL–AF9 target genes was reduced in these cells, although there were some exceptions. DOT1L occupancy of *Hoxa7–10* was reduced in *Af10*$^{-/-}$ cells expressing MLL–AF9, whereas MLL–AF9 occupancy was unaffected. In addition, the DOT1L–AF10 complex had much higher activity than DOT1L alone in an *in vitro* histone methyltransferase assay.

Finally, the authors showed that NUP98–NSD1-transformed LSKs in which *Af10* was deleted had reduced H3K79me2 levels at the *Hoxa7–10* cluster and reduced proliferation. Similar results were obtained by treatment of these cells with EPZ004777, which significantly reduced *Hoxa7–10* expression (as well as H3K79me2 levels) and cell proliferation in a dose-dependent manner, indicating that some non-MLL-rearranged AMLs may also depend on DOT1L–AF10 for the regulation of HOXA gene expression.

This paper identifies a key role for the DOT1L complex cofactor AF10 in regulating progressive H3K79 methylation and shows that DOT1L–AF10 regulates HOXA gene expression in various subtypes of leukaemia, potentially increasing the opportunity for DOT1L inhibition.

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**ORIGINAL RESEARCH PAPER** Deshpande, A. J. *et al.* AF10 regulates progressive H3K79 methylation and HOX gene expression in diverse AML subtypes. *Cancer Cell* **26**, 896–908 (2014)