MLL5 expression as a biomarker for DNA hypermethylation and sensitivity to epigenetic therapy

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One challenge in cancer biology is to understand how molecular variability impacts patient response to therapy. Epigenetic changes are likely to be a major source of variability but can be difficult to identify. In this issue of Haematologica, Yun and colleagues show that high expression of the *Mixed Lineage Leukemia 5* gene (*MLL5*) predicts a better therapeutic response to decitabine (DAC), a DNA hypomethylating agent that can alter the epigenetic information content of the cell. They further show that MLL5 expression itself may sensitize the leukemia cell to DAC by increasing DNA methylation at gene promoters. As well as identifying a new biomarker that could be used to guide DAC therapies, this study also potentially links histone methylation with DNA methylation in leukemia cells.

Epigenetics: H3K4 methylation and DNA methylation

Relative to most other adult cancers, patients with acute myeloid leukemias (AML) have a surprisingly small number of potential driver mutations in their genome.[1] Even so, not all patients within a defined mutational subset necessarily respond the same way to therapy. One additional source of variability is due to epigenetic changes.[2] Epigenetics is generally defined as the study of gene expression patterns that are stably transmitted through the cell cycle due to the activity of non-coding RNAs, the covalent modification of histone proteins and their variants, or the covalent modification of DNA.[2] Different epigenetic 'marks' are associated with different functional outputs. For example, the addition of three methyl groups to lysine 4 on histone 3 (H3K4Me3) is strongly correlated with gene activation.[3] H3K4Me3 levels are controlled by lysine 4 specific methyltransferases that add the mark and lysine 4 specific demethylases that remove it.[3] Exciting research has recently suggested that one major role for H3K4Me3 may be to directly promote gene activation by stimulating the formation of the pre-initiation complex at gene promoters.[4] Genome wide analyses suggest that H3K4Me3 is associated with 'poised' as well as active gene promoters.[2]

Conversely, DNA methylation at promoters is generally associated with gene repression, most likely due to the recruitment of methyl-CpG binding domain (MBD) proteins.[2,5] DNA methylation of the 5th atom of cytosine (5mC) is initiated by the activity of the DNA methyltransferases 3A and 3B (DNMT3A/3B) and is maintained through the cell cycle by the activity of DNMT1.[5] Although DNA demethylation can occur through passive dilution, it is also directly controlled by ten-eleven translocation (TET) family proteins. TET1, 2 and 3 catalyze the conversion of 5mC to 5-hydroxymethyl C (5hmC), which can eventually result in the regeneration of an unmodified cytosine.[5] It is unknown what the direct function of 5hmC might be, but it is only rarely found at CpG rich promoters and tends to correlate with gene activation when found in the gene body.[5]

Yun *et al*. show that high expression of *MLL5*, a putative H3K4 methyltransferase, correlates with increased DNA methylation at gene promoters in leukemia cells. The potential functional consequences of this novel observation are discussed below.

MLL5 and H3K4Me3

The *MLL5* gene was originally cloned from a segment in band 7q22 that is commonly deleted in AML and MDS.[6] Due to domain similarities, MLL5 was originally proposed to be a functional member of the MLL family of proteins.[6] *Mll5* knockout mice display hematopoietic stem cell defects,[7-9] a phenotype also observed in *Mll* knockout models.[10,11] However, MLL5 does not appear to be a typical MLL family member.

The MLL family consists of 6 different members (MLL, MLL2, MLL3, MLL4, SET1A and SET1B), based on the high degree of similarity in their SET domains.[3] The SET domain has intrinsic H3K4 methyltransferase activity, although each protein also has other important functional domains.[3] Other than the SET domain, some members of the MLL family also contain highly similar plant homedomain (PHD) fingers. In MLL, PHD3 binds directly to H3K4Me3 (Figure 1A) and stabilizes MLL binding to target genes.[12]

MLL5 is generally found at actively transcribed genes marked with H3K4Me3.[13,14] Although MLL5 contains both a SET domain and a PHD finger (Figure 1A), the sequence of both domains diverge from the MLL family,[3] and the MLL5 SET domain does not appear to have intrinsic H3K4Me activity.[8,15] Despite this, MLL5 may control H3K4Me3 at gene targets through an indirect mechanism.[14] Although it differs in sequence from MLL family members,[3] the MLL5 PHD finger also binds directly to H3K4Me3.[13,16] Yun *et al*. have shown that MLL5 expression correlates with increased DNA methylation levels genome wide, while lack of MLL5 results in a genome wide decrease of DNA methylation at gene promoters. If the main role of MLL5 is to promote gene activation, this is a highly unexpected result.

Does MLL5 control DNA methylation?

Yun *et al*. use MeDIP coupled with promoter microarray analysis to assay genome wide changes in DNA methylation. In HOXA9/MEIS1 transformed *Mll5-/-* bone marrow cells, DNA methylation at gene promoters is generally reduced compared to HOXA9/MEIS1 transformed *Mll5+/+* bone marrow cells. They further show that high MLL5 expression correlates with a high degree of hypermethylation and only rarely with hypomethylation of gene targets in different AML patient samples.

Could MLL5 directly induce hypermethylation in leukemia cells? Like other MLL family members[17,18] (Figure 1), MLL5 interacts with the host cell factor (HCF) proteins, which in turn interact with O-GlcNAc transferase (OGT).[14] OGT interacts with TET2/3 proteins, all of which appear to be necessary for maintaining H3K4Me3 at some gene promoters.[19,20] Perhaps MLL5 somehow prevents HCF/OGT interactions with TET2/3 (Figure 1B), disrupting the conversion of 5mC to

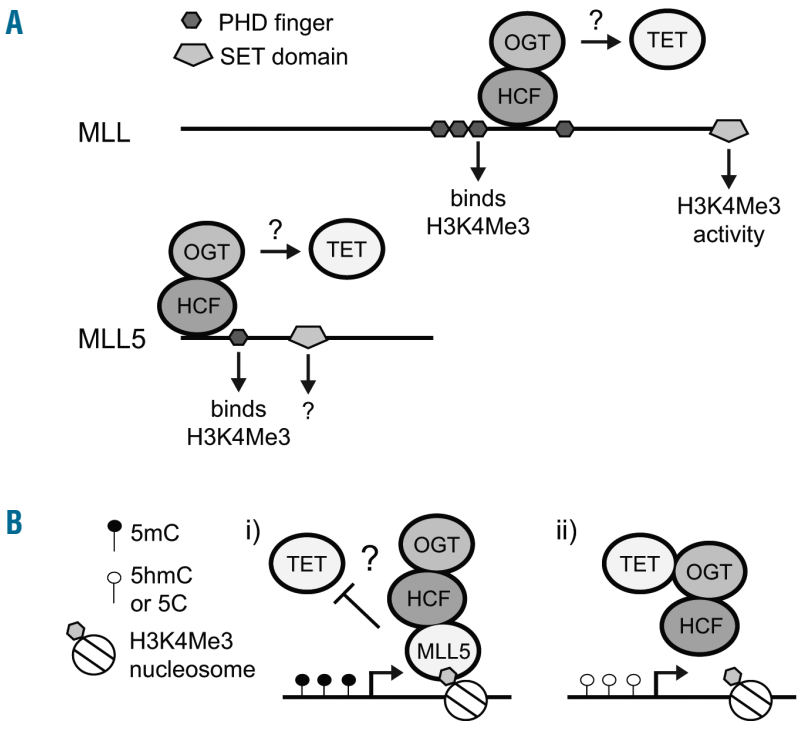

Figure 1. MLL5 activity. (A) The long form of the MLL5 protein is shown along with the relative positions of the PHD finger and the SET domain as compared to MLL. Both MLL and MLL5 bind directly to HCF proteins and indirectly with OGT. OGT can also interact with TET2/3 proteins. (B) If MLL5 can directly increase DNA methylation (5mC) at gene promoters, one possibility is that it binds to the HCF/OGT complex and inhibits TET protein activity (i). In the absence of MLL5, TET proteins would then be free to demethylate the promoter (ii).

5hmC, and thus increasing DNA methylation at gene promoters (Figure 1B). However, MLL5 is usually found bound to H3K4Me3 marked active genes,[13] gene targets that are not typically marked with 5mC,[5] making this model unlikely. It will be important to know if MLL5 binding actually correlates with increased 5mC at gene promoters. If MLL5 does control DNA methylation in the cell, it is more likely to be indirect. One simple possibility is that MLL5 activates DNMT1/3A/3B gene expression thus increasing DNMT protein levels and inducing higher 5mC levels globally. A complete understanding of this interesting observation by Yun *et al.* will await further detailed molecular analyses of MLL5 activity in leukemia cells.

MLL5 expression as a prognostic marker for decatibine sensitivity

Whatever the mechanism of MLL5-associated DNA hypermethylation, Yun *et al.* were able to show that, compared to patients expressing low *MLL5*, patients expressing high *MLL5* display a statistically significant increase in overall survival (OS) when treated with at least three courses of DAC. Consistent with the possibility that this increased OS is due to the higher methylation levels in patients expressing high *MLL5*, Yun *et al.* were also able to show that DNMT3A wild-type patients responded better to DAC than DNMT3A mutant patients. The increased sensitivity to DAC in the presence of high *MLL5* expression was recapitulated in two different cell culture leukemia model systems. More specifically, *Mll5-/-* bone marrow cells transformed with either HOXA9 alone or HOXA9 combined with MEIS1, were much less sensitive to DAC treatment than *Mll5+/+* transformed cells. Interestingly, they were also able to show that DAC treatment reduced DNA methylation levels to a greater degree in *Mll5+/+* HOXA9/MEIS1 cells than in *Mll5-/-* HOXA9/MEIS1 cells.

Together, these data establish a strong correlation between high *MLL5* expression, DNA hypermethylation, response to DAC treatment and improved OS.

Mechanism of MLL5 induced decatibine sensitivity in leukemia

How could MLL5-associated DAC sensitivity be working on a mechanistic level in leukemia? As discussed above, MLL5 expression itself may induce DNA hypermethylation in the cell. Leukemia cells expressing *MLL5* could thus become addicted to high levels of DNA methylation and have increased sensitivity to loss of DNA methylation via DAC treatment.

Another possibility suggested by Yun *et al.* is that perhaps high *MLL5* expression simply correlates with differentiation. This would mean that higher relative methylation levels as well as increased sensitivity to DAC may simply be due to the presence of more differentiated cells in a particular leukemia. This idea is partly supported by the observation that *Mll5* expression increases with myeloid differentiation.[7] Although a full answer awaits further molecular analysis, Yun *et al.* have shown that high *MLL5* expression could be a useful marker for guiding future therapeutic strategies.

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Treating advanced cardiac damage in light chain amyloidosis: still an unmet need

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In this issue of the Journal, Jaccard *et al.* report the outcome of a retrospective multi-institutional experience with bortezomib, cyclophosphamide and dexamethasone (VCD, or CyBorD) used as up-front therapy in 60 Mayo stage III treatment naïve patients with systemic light chain amyloidosis (AL amyloidosis).[1] Since cardiac damage rapidly progresses in this disease, the authors evaluated if this regimen, which is known to act rapidly inducing a substantial proportion of profound hematologic responses, was able to rescue cardiac function and extend survival in a population with advanced heart damage. In a multicenter setting, VCD produced an overall response rate of 68% with 42% of patients achieving a very good partial response or a complete response that translated into 32% cardiac responses. Cardiac response was predictive of survival with an estimated, remarkable, 1-year overall survival of 89% for responders. However, 24 patients (40%) died while on therapy and in patients with very advanced amyloid cardiac damage, identified uniquely by high values of the cardiac biomarker N-terminal pro-natriuretic peptide type-B (NT-proBNP) or BNP (NT-proBNP >9500 ng/L or BNP >1100 ng/L), the outcome was dismal with a median survival of only 4.4 months. The results open up a wide array of considerations and perspectives.

Cardiac damage is the determinant of survival in virtually all patients with AL amyloidosis.[2] Patients who present with advanced heart involvement, defined by very high levels of NT-proBNP, survive only a few months, representing the major impediment to a further improvement in life expectancy in this disease.[3] Clinical and experimental evidence indicate that cardiac damage in AL amyloidosis is mainly determined by a direct toxicity exerted by the circulating amyloidogenic free light chain (FLC). Thus, therapy for cardiac amyloidosis is aimed at obtaining a rapid and profound FLC reduction by targeting the amyloidogenic plasma cell clone with chemotherapy. Several groups have investigated this problem with studies evaluating the efficacy of front-line conventional and novel therapies in patients with amyloid cardiomyopathy.[1,2] The outcome of these studies is disheartening, with 20-40% early mortality rates and median survival spanning from a