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Myeloma's sound of silencing

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In this issue of *Blood*, Ren et al demonstrate that the polycomb repressive complex 2 (PRC2)–associated protein PHF19 activates PRC2-induced histone 3 lysine 27 trimethylation (H3K27me3) to promote multiple myeloma cell growth and tumorigenesis.[1] These findings provide new evidence for why myeloma cells are dependent on PRC2 and add support for therapeutic targeting of this epigenetic regulator in this disease.

There is overwhelming evidence that cell fate decisions are controlled through the epigenetic modulation of gene expression.[2] This includes the silencing of genes to "lock" the cell into a differentiated state. Thus, it is not surprising that alterations in the machinery that controls cell differentiation play an important role in cancer development and progression. The plasma cell malignancy multiple myeloma is no exception, having mutations in epigenetic regulators. This includes overexpression of a truncated form of the histone 3 lysine 36 (H3K36) methyltransferase NSD2 (WHSC1 or MMSET), which results from t(4;14), and is found in 15% to 20% of myeloma patients. The H3K27me3 demethylase KDM6A (UTX) is also mutated or deleted in a small fraction of myeloma cell lines and patient samples.[3] However, mutations cannot completely explain the dysregulation of epigenetic control in myeloma. Early studies demonstrated repression of genes that were known targets of PRC2 in myeloma,[4] and subsequent studies showed that the product of PRC2 activity, H3K27me3, was increased in myeloma compared with normal cells and associated with poor outcomes.[5] PRC2 is made up of a core complex, including a catalytic subunit (EZH1 or EZH2), an epigenetic reader protein that can bind to H3K27me3 to facilitate spreading of this repressive mark (EED), and a scaffold protein (SUZ12).[2] The activity of the complex is regulated by accessory proteins, such as the histone binding protein (RBBP4/7), as well as regulatory proteins, including the polycomb-like proteins (PCL1/PHF1, PCL2/MTF2, PCL3/PHF19), JARID2, and AEBP. Recent studies have shown that these accessory proteins likely function in recruitment and stabilization of PRC2 to assure efficient H3K27me3 at silenced genes. This makes these "activators" of PRC2 a potential site of oncogenic dysregulation. Unfortunately, very little is known about their role in myelomagenesis, with the notable exception of JARID2 being identified as a susceptibility locus in a genome-wide association study of myeloma[6] and PCL2/MTF2 being in a frequently deleted region of chromosome 1p.[7] Based on high levels of expression in myeloma cell lines, the focus of the current study was on PHF19.

The investigators showed that expression of PHF19 increased with disease progression through comparison of messenger RNA levels in plasma cells from healthy individuals and patients at various stages of progression. They also showed that PHF19 was the highest expressed PRC2 accessory factor in myeloma, suggesting that it likely plays a role in the disease pathogenesis; importantly, expression was associated with poor outcomes in several trials. Consistent with these findings, silencing of PRF19 in myeloma cell lines resulted in decreased cell growth, colony formation, and tumor growth. These findings are similar to published studies of EZH1/2 inhibitors.[8]

PRC2 catalyzes the methylation of H3K27, and previous reports suggested that PHF19 functions as a reader of histone 3 lysine 36 trimethylation (H3K36me3).[9] H3K36me3 is associated with actively transcribed genes; therefore, PHF19 targeting of PRC2 to this histone mark has been proposed as a means to silence activated genes. The investigators identified changes in H3 methylation following PHF19 silencing. Consistent with its role as an activator of PRC2, silencing PHF19 resulted in a decrease in H3K27me3, and chromatin immunoprecipitation sequencing data demonstrated that this occurred throughout genes that were transcriptionally repressed in parental cells. This is consistent with a model of H3K27me3 spreading, whereby PRC2 is initially nucleated and then can move along the chromatin through an allosteric activation event.[2] The EED core component can bind to H3K27me3

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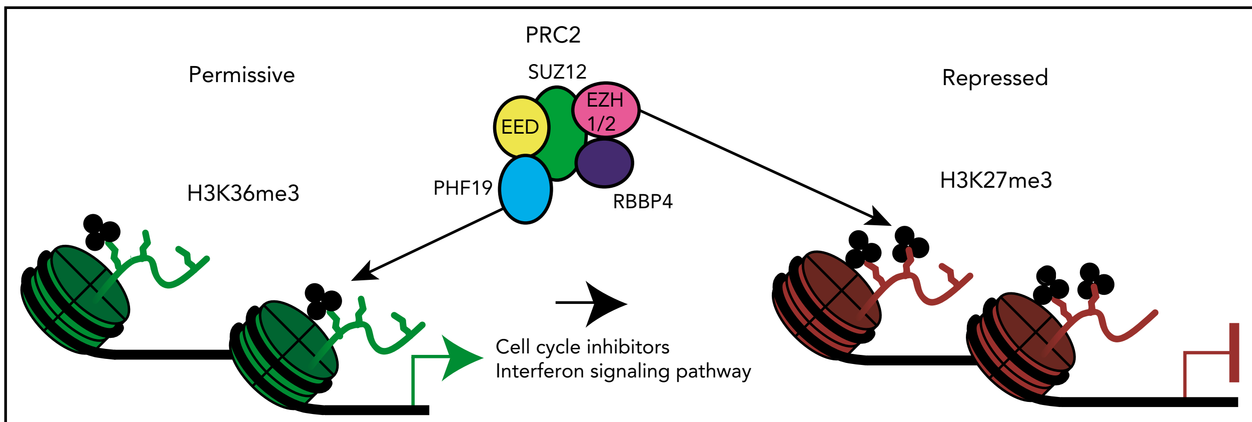

Model of PHF19 regulation of transcriptional silencing in myeloma. Potentially through its recognition of H3K36me3 in active genes (green), PHF19 recruits PRC2 to methylate H3K27, resulting in gene silencing.

on 1 nucleosome, resulting in a conformational change in the complex that allows EZH1/2 to methylate H3K27 on the next nucleosome, thereby spreading the histone mark.[10] Interestingly, the effects of PHF19 silencing on H3K27me3 were only observed at regions outside of CpG islands (CGIs), suggesting that the maintenance of H3K27me3 at CGIs occurs through a different mechanism. Regardless, the loss of H3K27me3 results in the activation of genes that have previously been shown to be PRC2 targets in myeloma, specifically genes associated with inhibition of the cell cycle (eg, CDKN1C) and interferon signaling (eg, STAT5A). Analysis of patient gene expression data confirmed that PHF19 expression negatively correlated with expression of genes that were bound by H3K27me3.

Finally, the investigators used PHF19-mutant alleles to determine how they controlled PRC2 activity and cell and tumor growth in myeloma. First, they showed that PHF19 mutants that cannot associate with the PRC2 core complex cannot rescue cells from PHF19 silencing. Then they deleted the Tudor and EH domains, which are involved in binding to chromatin, and found that loss of either domain also could not rescue the effects of PHF19 silencing on cell/tumor growth and/or gene expression. Although important, this finding does raise a question about the proposed mechanism. The Tudor domain is important for H3K36me3 binding, consistent with a role for this mark in PHF19-dependent recruitment of PRC2 (see figure). However, it is unclear why deletion of the EH domain, which is involved in binding to unmethylated CGI DNA, would have an effect. Thus, the mechanism of PHF19-mediated PRC2 recruitment/activation may be more complicated. Moreover, the current study does not formally demonstrate a role for H3K36me3 in the recruitment of PRC2; however, if H3K36me3 is important for PHF19 regulation of PRC2, then it would be interesting to determine how t(4;14) influences PRC2 activity in myeloma.

In addition to shedding new light on how PRC2 is activated/regulated in myeloma, the current study points to a potentially more myeloma-selective means to therapeutically target PRC2. Because PHF19 is upregulated and important for PRC2 activity in myeloma, targeting PHF19 could provide a selective approach to target this important regulator of myeloma pathogenesis.

Conflict-of-interest disclosure: L.H.B. declares no competing financial interests. ■

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