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Oncogene interference through targeting of chromatin regulators

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The chromatin regulatory machinery is often exploited during tumorigenesis.[1] For example, many of the central transcription factors in cancer biology (such as p53 and Myc) utilize chromatin regulators (CRs) as coactivators or corepressors to regulate their downstream target genes. Moreover, somatic mutation of CR-encoding genes is a common driver mechanism seen in many cancer types.[2] Hence, CRs often govern gene expression programs that endow cancer cells with their hallmark capabilities. Since CRs also possess desirable biochemical properties for the design of small-molecule inhibitors, therapeutic targeting of CRs provides a rational approach for interfering with the aberrant signaling events that drive cancer progression.

A major challenge in developing CRs as drug targets lies in determining whether cancer cells harbor unique vulnerabilities among this class of regulators. The majority of CR gene mutations identified in cancer are loss-of-function, hence they do not provide straightforward opportunities for targeting. In addition, most CRs are ubiquitously expressed proteins with broad chromatin occupancy, properties that confuse evaluating their suitability as targets. To explore these issues, we have taken a genetic screening approach toward identifying CR vulnerabilities in the blood cancer acute myeloid leukemia (AML); specifically in the subset of disease caused by rearrangements of the mixed-lineage leukemia (*MLL)* gene. *MLL* is mutated via chromosomal translocation in ~5% of AML patients, resulting in the generation of chimeric fusion proteins.[3] The wild-type form of *MLL* encodes a histone H3K4 methyltransferase, however MLL-fusion proteins found in leukemia have lost this CR activity and instead alter gene expression via their C-terminal fusion partner. While MLL is known to fuse with diverse proteins to promote leukemogenesis, many of its common fusion partners interact with the histone H3K79 methyltransferase DOT1L. By hijacking DOT1L to its target genes, MLL-fusion proteins deregulate gene expression to promote leukemia formation.[3] The reliance of MLL-fusion leukemia on DOT1L provides one of the clearest examples of a CR vulnerability imposed by the presence of a specific oncogene.[4] Using negative-selection shRNA screens focused on CRs, we have identified several additional vulnerabilities present in MLL-rearranged leukemia, such as the BET bromodomain protein BRD4 and the Polycomb complex PRC2.[5,6] Importantly, both represent vulnerabilities that can be targeted with small-molecule inhibitors and, hence, are candidate drug-targets in this disease.

Histone mono-ubiquitination is a fundamental chromatin modification that regulates transcription, however its involvement in the pathogenesis of cancer is not well established. To explore the involvement of histone mono-ubiquitination in maintenance of MLL-fusion leukemia, we performed a negative-selection shRNA screen directed at all known regulators of this modification.[7] This screen identified only a single "hit": RNF20, which is an E3 mono-ubiquitin ligase specific for H2B lysine 120. RNF20 knockdown via shRNA triggers a severe impairment in MLL-fusion leukemia proliferation, correlating with global reductions in H2BK120 mono-ubiquitination (H2Bub). By introducing RNF20 shRNAs into a variety of heterologous cell types (fibroblasts, epithelial cancer lines, immortalized hematopoietic lines), we found that the hypersensitivity of MLL-fusion leukemia to RNF20 inhibition is highly unique to this cellular context.[7] Experiments performed in human AML cell lines further confirmed that growth of cells with MLL-rearrangements is hypersensitive to RNF20 inhibition as compared with lines without this translocation.[7]

Prior studies have shown that H2Bub stimulates the catalytic activity of DOT1L.[8] Given the essential role of H3K79 methylation (H3K79me) in MLL-fusion leukemia, we considered that RNF20 and DOT1L might operate in the same pathway to support leukemia maintenance. Strikingly, the global gene expression changes incurred upon RNF20 knockdown closely match those seen following DOT1L suppression.[7] Moreover, levels of H2Bub and H3K79me globally correlate with one another across the leukemia genome.[7] Finally, we found that suppressing RNF20 expression leads to reduced H3K79me levels in the gene body of MLL-fusion target genes, but not globally across the genome.[7] Together, these findings support RNF20 as a component of a chromatin-based signaling pathway that emanates from the MLL-fusion oncogene to regulate transcription. In our model, RNF20 acts in a positive-feedback loop that couples H2Bub to transcription elongation induced by MLL-fusion proteins. The presence of H2Bub enhances the activity of DOT1L, thereby augmenting transcriptional activation by the MLL-fusion protein (**Fig. 1**).

These results illustrate how blockade of an oncoprotein can be achieved through targeting of CRs that operate in the same regulatory pathway. While our genetic evidence places RNF20 in the same

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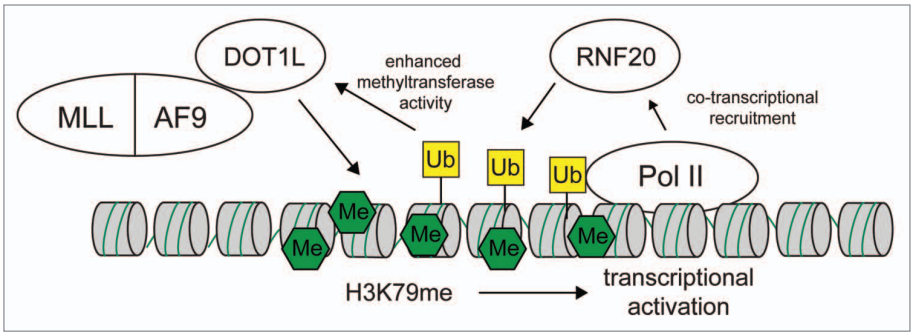

Figure 1. RNF20 supports the pathogenesis of MLL-fusion leukemia by amplifying DOT1L-mediated H3K79 methylation. RNF20 is likely to be recruited to MLL-fusion target genes via interactions with the PAF transcription elongation complex, which physically interacts with RNA polymerase II. Ub, H2BK120 mono-ubiquitination; Me, H3K79 methylation. Illustration depicts events occurring at MLL-fusion target genes, such as *Hoxa9* and *Meis1*.

pathway as MLL-fusions and DOT1L, it should be pointed out that RNF20 is also globally coupled to RNA polymerase II at all actively transcribed genes.[7] Indeed, levels of H2Bub are not particularly elevated at MLL-fusion target genes as compared with other active sites in the genome.[7] Nevertheless, the expression of MLL-fusion target genes is uniquely dependent on RNF20 as compared with other genes. The full mechanistic basis for this hypersensitivity remains to be determined, however, the reliance on RNF20 for gene expression correlates with a necessity for RNF20 to support local H3K79me by DOT1L. Perhaps the turnover rate of H3K79me at MLL-fusion target genes is higher than elsewhere in the genome, thus imposing an RNF20 requirement to achieve sufficient DOT1L activity. Collectively, our studies illustrate how genetic screening approaches can reveal actionable connections between CRs and driver oncoproteins, particularly those that have evaded attempts at direct targeting.[5-7]

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