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PAF Is in the Cabal of MLL1-Interacting Proteins that Promote Leukemia

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MLL1 fusions are among the most potent oncogenic drivers of leukemia development. In recent articles published in *Molecular Cell* and in *Cancer Cell*, researchers find that MLL1 fusions are reliant on a physical interaction with the PAF transcription elongation complex for their recruitment to chromatin and, consequently, leukemic transformation.

Acute myeloid or lymphoid leukemias harboring rearrangements of the MLL1 gene represent a poor-prognosis subset of these diseases with a general unresponsiveness to chemotherapy (reviewed in Krivtsov and Armstrong, 2007). Chromosomal translocations that disrupt MLL1 generate oncogenic gene fusions encoding the MLL1 N-terminal region fused to one of a variety of different partner proteins (>50 are known). A consistent feature of otherwise diverse fusion partners is the corruption of MLL1's normal capacity to promote self-renewal of hematopoietic cells. The resulting MLL1 fusion undermines normal differentiation pathways to immortalize hematopoietic cells in an immature state. While evidence from mouse models has established MLL1 fusions as among the most potent drivers of leukemia known, effective strategies have yet to be identified for neutralizing leukemic MLL1 functions for therapeutic benefit. One avenue toward identifying novel therapeutic handles in these aggressive leukemias is to elucidate the essential biochemical framework of MLL1 fusion protein complexes. Two recent articles published in *Cancer Cell* and *Molecular Cell* have made a pivotal advance in this regard by identifying a specific interaction between the PAF complex and MLL1 that is required for leukemic transformation (Milne et al., 2010; Muntean et al., 2010). Hence, PAF is exposed as a conspirator that, along with two other MLL1-associated proteins, Menin and LEDGF, promotes leukemogenesis conferred by MLL1 fusion proteins.

MLL1 performs its normal and leukemic functions through involvement with active chromatin states (Krivtsov and Armstrong, 2007). Like many other chromatin regulators, MLL1 is composed of an assortment of domains (AT hooks, CXXC, BROMO, PHD) that can latch onto DNA or histones, as well as a catalytic SET domain at the C terminus that methylates histone H3 at lysine 4, a modification implicated in active transcription (Milne et al., 2002). MLL1 also has been shown to interact with numerous proteins to form a higher-order complex, e.g., Menin, LEDGF, HCF-1, ASH2L, RbBP5, and WDR5 (Yokoyama et al., 2004). In contrast to the full-length molecule, the MLL1 fragment present in leukemogenic fusions only retains the Menin/LEDGF interaction domain, AT hooks, and the CXXC domain, which together are sufficient for recruitment to target sites in the genome. Distortion of MLL1 function is due to the replacement of its native C-terminal effector domains with those provided by one of many C-terminal fusion partners. Indeed, a large number of studies have identified protein complexes associated with many of the most common MLL1 fusion partners (ENL, AF4, and AF9), all of which seem to share a group of factors linked with regulating transcription elongation, e.g., pTEFb and the histone methyltransferase DOT1L (e.g., Mueller et al., 2007). Thus, MLL1 fusions assemble a multisubunit complex of transcriptional regulators that leads to altered expression of MLL1's normal target genes, such as HOXA9.

A major mechanistic question addressed in the articles by Muntean et al. (2010) and Milne et al. (2010) regards the recruitment mechanism employed by the

MLL1 portion of the fusion molecule. Prior work had established the interaction between MLL1 and Menin/LEDGF at the extreme N terminus and the presence of the CXXC domain as the most critical regions of MLL1 needed for leukemic transformation (Ayton et al., 2004). Whereas LEDGF may promote MLL1 fusion recruitment via an affinity for chromatin via its PWWP domain, the MLL1 CXXC domain has a selective affinity for unmethylated CpG DNA that could allow MLL1 fusion occupancy at promoter regions (Ayton et al., 2004). However, evidence suggested that CpG DNA binding alone cannot explain the requirement for MLL1's CXXC domain in leukemogenesis and that an additional unidentified activity/interaction conferred by this domain must be involved (Bach et al., 2009). This prompted the authors of both studies to examine whether a region encompassing the CXXC domain and flanking sequences interacts with other proteins by immunoprecipitating this specific fragment and identifying interacting proteins by mass spectrometry. Both studies identified the multisubunit PAF (polymerase associated factor) complex as an interacting partner of the extended MLL1 CXXC region and revealed this interaction as essential for leukemogenesis.

PAF was first identified as a complex that associates with RNA polymerase II and is composed of Paf1, Cdc73, Leo1, Ctr9, Rtf1, and Ski8 (reviewed in Chaudhary et al., 2007). PAF has been implicated in a range of regulatory activities, including transcription elongation, mRNA processing, H3K4 and H3K79 methylation, and H2B ubiquitylation. Although no prior studies had implicated the PAF complex in the pathogenesis of leukemia, Cdc73 is known to function as a tumor suppressor in parathyroid cancers—an interesting parallel with the MLL1-interacting protein Menin, which is also a tumor suppressor in a host of endocrine cancers.

To determine the functional impact of PAF binding by MLL1, Milne et al. (2010) utilized published NMR structures of the MLL1 CXXC domain to design specific point mutations that selectively disrupted either its DNA-binding activity or the PAF interaction. Both mutations led to defects in MLL1-AF9 recruitment to HOXA9 in vivo, with the compound disruption of both interactions abolishing recruitment. The authors then introduced the same mutant forms of MLL1-AF9 into bone marrow cells and determined that both DNA and PAF binding are independently required for efficient cell immortalization. Milne et al. (2010) also provided a mechanistic explanation for the recently published findings that wild-type MLL1 is required for the leukemogenic activity of MLL1 fusion proteins (Thiel et al., 2010). They show that the third PHD domain of MLL1, which "reads" H3K4me2/3 marks on histones, is essential for recruiting wild-type MLL1 to the HOXA9 locus. The PHD domains, however, are missing from the leukemogenic MLL fusion proteins, and therefore the MLL1-AF9 fusion protein cannot be recruited to the HOXA9 locus by itself (i.e., in the absence of wild-type MLL1). When wild-type MLL1 is present, however, MLL1-AF9 can occupy the HOXA9 locus. The authors conclude that wild-type MLL1 must first occupy the HOXA9 locus and create an open chromatin state that subsequently enables MLL1-AF9 to bind.

Muntean et al. (2010) likewise performed a detailed deletion analysis to identify two contact points between sequences flanking the MLL CXXC domain and the Paf1/Ctr9 proteins. The authors went on to demonstrate that disrupting the MLL1-PAF interaction both via mutation and by knocking down expression of Ctr9 or Cdc73 can inhibit MLL1-leukemia growth by abrogating MLL1 recruitment to Hox genes. Additionally the authors noted that PAF subunit expression is dynamically regulated during hematopoiesis, such that PAF might have important functions in normal hematopoiesis in controlling stemness, similar to what has been observed in embryonic stem cells.

Several additional questions remain to be answered regarding PAF's role in MLL1 function and the therapeutic potential of targeting this interaction. A major issue is the relative importance of the MLL1-PAF1 interaction for normal versus malignant functions of MLL1. Wild-type MLL1 is required to maintain normal hematopoiesis, so it remains to be seen whether MLL1 leukemias are more "addicted" to PAF complex function than their normal counterparts, an issue that could be addressed via conditional knockouts of PAF subunits in mice. The two articles also differ in their mapping of the precise interacting sites between MLL1 and PAF (RD1 versus RD2 region); therefore, solving the structure of an MLL1-CXXC-PAF complex would clarify the precise interaction surface among these molecules. Another important area of future investigation will be to determine whether PAF is simply a mode of recruitment for MLL1 or whether it also participates in chromatin modification at MLL1-bound sites. To this end, PAF has been shown to bind the BRE1 E3 ligase that catalyzes H2B ubiquitylation, a histone mark known to stimulate DOT1's methyltransferase activity for H3K79 (reviewed in Shilatifard, 2006). Several possibilities remain to be examined for how precisely PAF interfaces with the complex regulatory mechanisms employed by MLL1 fusion proteins in leukemia.

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