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CHAF1B Overexpression: A Brake for the Differentiation of Leukemia Cells

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In this issue of *Cancer Cell*, Volk et al. report that overexpression of CHAF1B displaces myeloid transcription factors from chromatin, and deletion of CHAF1B promotes differentiation of leukemia cells and suppresses leukemogenesis in a murine model, revealing a causal role of and an unexpected mechanism for CHAF1B overexpression in tumorigenesis.

Chromatin replication during S phase of the cell cycle is critical for the maintenance of the chromatin state, and yet it also provides a window of opportunity for orchestrating changes in the chromatin landscape in response to environmental and developmental cues. DNA synthesis catalyzed by DNA polymerases in the chromatin environment requires the disassembly of the nucleosome, the basic repeat unit of chromatin, ahead of DNA replication forks. Following DNA replication, the duplicated DNA is reassembled into nucleosomes to maintain chromatin landscape and gene expression states via two distinct pathways: the transfer of parental histones and the *de novo* deposition of newly synthesized histones. In recent years, it has become increasingly clear that parental and newly synthesized H3-H4 tetramers form distinct nucleosomes and that nucleosome assembly of parental and new H3-H4 tetramers is regulated via distinct mechanisms. This dynamic regulation of nucleosome assembly via distinct pathways ensures the transmission of epigenetic information into daughter cells and, at the same time, provides a regulatory window for alterations in chromatin states (Ransom et al., 2010; Serra-Cardona and Zhang, 2018).

Chromatin Assembly Factor 1 (CAF-1) is a histone chaperone conserved from yeast to human cells involved in the deposition of newly synthesized H3-H4 tetramers. CAF-1 consists of three subunits, which in mammalian cells are encoded by *CHAF1A*, *CHAF1B*, and *RBAP48*, respectively. CAF-1 interacts with proliferating cell nuclear antigen (PCNA), a DNA polymerase clamp for DNA polymerases, and co-localizes with PCNA at replication foci in early S phase of the cell cycle, consistent with the idea that CAF-1 is recruited to DNA replication forks through its interaction with PCNA for nucleosome assembly. In addition to its classic role in DNA replication-coupled nucleosome assembly, CHAF1A also interacts with heterochromatin protein 1 (HP1), and this interaction is required for pericentric heterochromatin replication and important for epigenetic silencing (Ransom et al., 2010; Serra-Cardona and Zhang, 2018). Moreover, CAF-1 is important to maintain the identity of somatic cells as deletion of CAF-1 enhances reprogram efficiency (Cheloufi et al., 2015). Thus, CAF-1 likely has multiple functions, most of which are linked to its role in DNA replication-coupled nucleosome assembly.

Given the important roles of CAF-1 in chromatin replication, it is not surprising that CAF-1 is altered in various cancers. For instance, overexpression of CHAF1B has been detected in several solid tumors, and increased expression of CHAF1B is associated with increased tumor grade and aggressiveness (Polo et al., 2010). Moreover, CHAF1B is highly expressed in acute megakaryocystic leukemia with Down syndrome (Malinge et al., 2012). However, it has remained unclear whether the CHAF1B overexpression has a causal role in tumorigenesis or is just a consequence of the tumor cell proliferation. In this issue of *Cancer Cell*, Volk et al. (2018) first showed that CHAF1B is essential for mouse hematopoiesis and cell viability by conditionally deleting CHAF1B in interferon-responsive cells, which is consistent with previous observations that CAF-1 is essential for mouse viability. Next, they overexpressed CHAF1B in hematopoietic stem and progenitor cells (HSPCs) and found that increased CHAF1B expression leads to increased proliferation of HSPC. To determine whether the increased proliferation has any role in leukemogenesis, they overexpressed CHAF1B in MLL-AF9 leukemia cells, a murine model of acute myeloid leukemia (AML), and found that increased CHAF1B expression leads to increased colony formation and enrichment of less differentiated colonies. Moreover, mice transplanted with the CHAF1B overexpressing MLL-AF9 cells die significantly faster than those transplanted with control MLL-AF9 cells without CHAF1B overexpression. In a set of reciprocal experiments, they found that deletion of CHAF1B in MLL-AF9 cells substantially reduces colony formation ability *in vitro* and prevents leukemogenesis in mice. More interestingly, they found that overexpression of a dominant-negative CHAF1A mutant, which binds CHAF1B and cannot be recruited to DNA replication forks due to defects in PCNA binding, blocks leukemia formation in the MLL-AF9 mouse model and colony formation of human AML cell lines, while having no apparent effect on the proliferation and differentiation of HSPCs *in vitro* and *in vivo*. Together, these results indicate that overexpression of CHAF1B most likely plays a causal role in leukemogenesis and that targeting the CAF-1-mediated nucleosome assembly pathway

provides an opportunity for the treatment of leukemia.

As CHAF1B functions in DNA replication-coupled nucleosome assembly, one would have expected that depletion of CHAF1B reduced proliferation of leukemia cells. Remarkably, they found that reducing the dosage of CHAF1B induces differentiation of both murine MLL-AF9 as well as human AML cells. Consistent with this observation, analysis of transcriptome of cells with both CHAF1B overexpression and deletion indicates that CHAF1B is important to silence a group of genes involved in myeloid differentiation. CHAF1B ChIP-seq results indicate that CHAF1B co-localizes with chromatin loci bound by CEBPA, FLI1, and RUX1, key myeloid differentiation transcription factors, and deletion of CHAF1B results in increased occupancy of these transcription factors at chromatin loci close to genes regulating differentiation. Together, these results support a model that increased dosage of CHAF1B competes with key myeloid differentiation transcription factors for chromatin binding, thereby silencing genes important for differentiation of leukemia cells. Supporting this idea, depletion of CEBPA partially restores the leukemogenic capacity of *Chaf1b* deleted cells. In another surprising twist, through a set of complementation assays using CHAF1B mutants that are defective in DNA replication-coupled nucleosome assembly, Volk et al. (2018) show that the classic function of CHAF1B in DNA replication-coupled nucleosome assembly is important for its role to block differentiation of leukemia cells.

Based on these results, Volk et al. proposed that CHAF1B overexpression drives leukemogenesis by participating in DNA replication-coupled nucleosome assembly and by competing with key myeloid differentiation transcription factor such as CEBPA for chromatin binding. While CHAF1A contains a DNA binding motif, it is unlikely that CAF-1 binds to DNA in a sequence-dependent manner. How, then, does one reconcile these two seemingly distinct functions of CHAF1B in leukemogenesis? Recent studies on nucleosome assembly of parental and newly synthesized H3-H4 tetramers may provide a clue. Several studies indicate that nucleosomes formed with parental H3-H4 tetramers most likely assume the original nucleosomal positions following DNA replication (Gan et al., 2018; Reverón-Gómez et al., 2018). In contrast, nucleosomes formed with newly synthesized H3-H4 tetramers, mediated by CAF-1 complex, are less stable at replicating chromatin and fill the gaps left behind by parental histones (Yu et al., 2018). Moreover, it has been shown that nucleosomes on newly replicated chromatin compete with transcription factors (Ramachandran and Henikoff, 2016). Therefore, it is possible that CAF-1-mediated nucleosome assembly of new H3-H4 tetramers at key myeloid differentiation transcription factor binding sites blocks these transcription factors from binding, thereby inhibiting expression of genes involved in differentiation of leukemia cells (Figure 1).

In summary, Volk et al. demonstrate that CHAF1B overexpression is critical for leukemogenesis, at least in part through blocking differentiation of leukemia cells. Targeting the CAF-1-mediated nucleosome assembly in leukemia cells with high levels of CHAF1B may provide a therapeutic strategy. It would be interesting to determine whether overexpression of CHAF1A and CHAF1B in solid tumor also has a similar role in tumorigenesis.

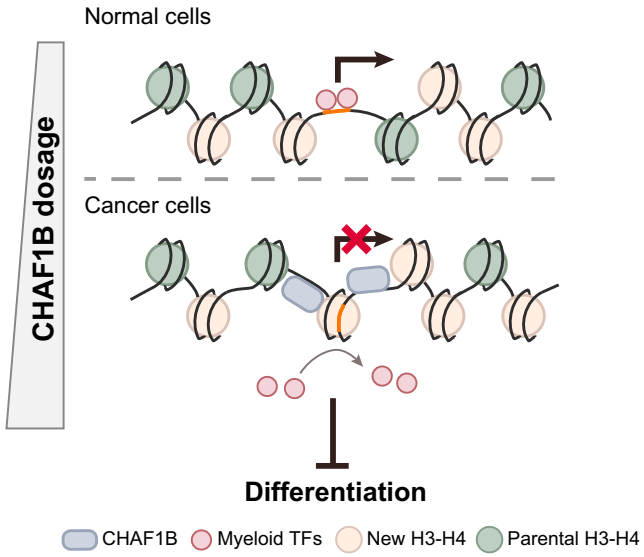

Figure 1. A Model for CHAF1B Overexpression in Leukemogenesis
TF, transcription factors. CHAF1B, shown in the figure, is known to form with CHAF1A and RBAP48 a complex that assembles new H3-H4 tetramers into nucleosomes following DNA replication.

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