Reconstructing the Disease Model and Epigenetic Networks for MLL-AF4 Leukemia

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The lack of a proper animal model has impeded understanding of the molecular mechanism of leukemia associated with the MLL-AF4 fusion. In this issue of *Cancer Cell*, Krivtsov et al. report a much-improved murine Mll-AF4 model and propose a molecular link with H3K79 methylation mediated by the histone methyltransferase DOT1L.

The mixed-lineage leukemia (*MLL*) gene, which encodes a histone methyltransferase, can rearrange with more than 50 different fusion partners in multiple-lineage leukemias, i.e., acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), and acute biphenotypic leukemia (ABL). While mouse modeling has been extremely successful in reconstructing myeloid disease, progress in developing ALL and ABL models for MLL has been rather limited.

MLL fusions such as MLL-ENL can target either committed murine myeloid progenitors (common myeloid progenitors or granulocyte macrophage progenitors) or hematopoietic stem cells (HSCs) to induce AML but fail to give rise to ALL or ABL (Cozzio et al., 2003). Multilineage leukemia has been achieved by targeting multipotent HSCs, but not myeloid progenitors, using MLL-GAS7, suggesting that both the origin of the leukemic stem cells and the MLL fusion partners play important roles in determining the disease phenotype (So et al., 2003). An instructive role of fusion partners in directing lineage specificity is further supported by findings from translocator models, where T lymphoid to myeloid lineage reassignment was observed only in cells carrying MLL-ENL, but not MLL-AF9, even though both were expressed under the same conditions (Drynan et al., 2005).

An animal model for the most common MLL fusion, MLL-AF4, which uniquely associates with ALL/ABL, is still missing. Using conventional knockin or conditional invertor approaches, Mll-AF4 is capable of inducing lymphoid malignancy with protracted latency (Chen et al., 2006; Metzler et al., 2006). However, the disease is predominately limited to mature B cell lymphoma, which is very different from pro-B ALL/ABL in humans. The lack of proper MLL-AF4 disease models has significantly impeded progress in understanding the underlying mechanisms for this fusion.

Reconstructing a MLL-AF4 Model and Its Corrupted Epigenetic Networks

In this issue of *Cancer Cell*, Armstrong and colleagues establish a much-improved Mll-AF4 disease model using a conditional expression approach wherein the human AF4 cDNA is inserted into the murine *Mll* exon 8, downstream of a transcriptional stop site flanked by *LoxP* sites, providing important molecular insights into MLL-AF4 pathogenesis (Krivtsov et al., 2008). They demonstrate that bone marrow cells expressing Mll-AF4 after retroviral Cre transduction have enhanced serial lymphoid replating ability and can induce multilineage leukemia following bone marrow transplantation.

Multilineage leukemia also occurred in this model when Mll-AF4 was conditionally expressed by crossing *Mll-AF4* mice with *Mx1-Cre* mice. Over half of these mice developed AML. However, about 40% of the mice developed B cell ALL, in which the majority were $B220^{+}CD19^{+}Mac1^{-}$ with clonal DJ_H rearrangements, with a few cases of pro-B cell-like ALL ($B220^{+}CD19^{-}Mac1^{-}$ with clonal DJ_H rearrangements). Interestingly, there were also three ABL cases co-expressing lymphoid (B220) and myeloid (Mac1) markers with or without immunoglobulin gene rearrangement. When comparing the frequency of leukemia-initiating cells (LICs) by limiting dilution transplant experiments, MLL-induced ALL had at least a 10-fold higher frequency of LICs than MLL-induced ABL.

The murine Mll-AF4-induced ALL model and human *MLL*-rearranged ALLs shared a similar gene expression signature and a significant enrichment of the H3K79me2 histone modification mark in the *HoxA* cluster and other specific loci as compared with their normal counterparts. Moreover, the specific H3K79me2 profile was also sufficient to distinguish human *MLL*-rearranged ALL from *MLL*-germline ALL. The H3K79me2 mark associated with active transcription is specifically conferred by the H3K79 methyltransferase DOT1L, which has previously been implicated in MLL-AF10 pathogenesis. When the expression of DOT1L in two different MLL-AF4 cell lines was downregulated using shRNAs, it reduced not only the H3K79me2 mark but also the expression of the *HOXA* cluster, which is believed to be a critical downstream target for MLL fusions. These results suggest that DOT1L may be a potential therapeutic target for MLL-AF4 leukemia.

Are We Missing the Real Cellular Targets?

The report by Armstrong and colleagues (Krivtsov et al., 2008) provides novel insights into MLL-AF4 pathogenesis but also raises two longstanding and unanswered questions: (1) What determines the lineage specificity of MLL, and (2) can we target epigenetic enzymes that associate with MLL fusions for leukemogenic suppression?

Although the Krivtsov et al. study provides the long sought-after B-ALL phenotype, MLL-AF4 in human leukemia almost exclusively associates with early pro-B or biphenotypic leukemia, which is quite different from the murine model, in which over half of the mice developed AML.

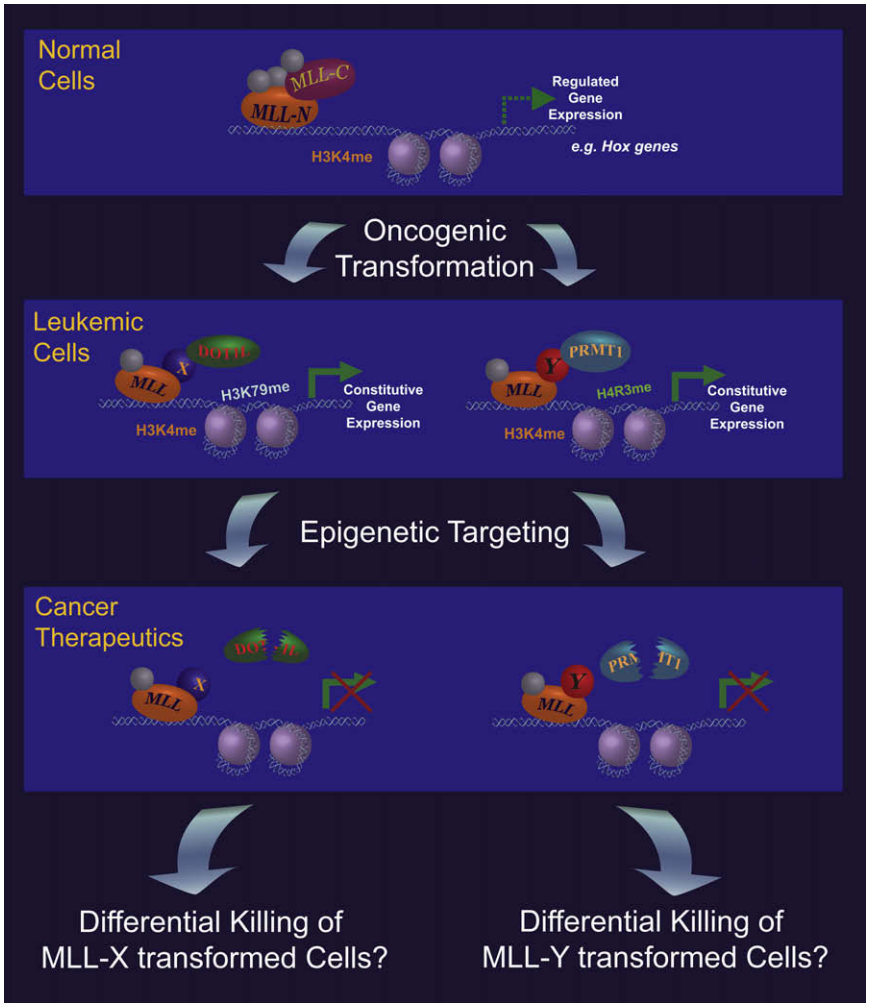

Figure 1. Epigenetic Targeting of MLL Leukemic Cells

Wild-type MLL (MLL-C and MLL-N) encoding a H3K4 histone methyltransferase mediates regulated gene expression via dynamic recruitment of transcription complexes. As a result of gene rearrangement in leukemia, MLL fusions (MLL-X and MLL-Y) recruit different transcriptional complexes containing new histone-modifying enzymes such as DOT1L (e.g., MLL-AF4) and PRMT1 (e.g., MLL-EEN), resulting in deregulated gene expression. It has already been shown that specific knockdown of these histone modification enzymes or abrogation of their catalytic activities inhibits MLL-mediated transformation. Although further studies are needed to determine the actual therapeutic window, development of small-molecule inhibitors targeting the rigid catalytic domain of these enzymes specifically recruited by MLL fusions represents a promising avenue for targeting these oncogenic transcription factors. (Illustration by Pui Yi Tse.)

Other unique features of human MLL-AF4 leukemia, including its remarkably short latency and specific association with *FLT3* deregulation/mutation, are not discussed in the report. However, the overwhelming number of AMLs argues against an instructive role of AF4 in defining the B-ALL phenotype. An alternative but not mutually exclusive explanation is that the MLL-AF4 model may have missed the "real targets." The majority of murine Mll-AF4 ALLs were more mature pre-B cell-like ALL, which is different from the human MLL-AF4 early pro-B ALL/ABL. It is not clear what cells (e.g., HSCs versus progenitors) were targeted in this MLL-AF4 model, as cellular fractionation experiments were not performed. However, the described multilineage leukemia is highly reminiscent of the one reported for MLL-GAS7 when multipotent HSCs were targeted (So et al., 2003), suggesting multipotent hematopoietic stem/progenitor cells as potential targets. Notably, the specific association of human MLL-AF4 ALL/ABL with neuron-glial antigen 2 (NG2) expression and the backtracking of MLL-AF4 clones to the prenatal stage suggest a very early developmental origin of the target cells (Gale et al., 1997). On the other hand, the microenvironment of the host may also influence the disease phenotype, as described recently for MLL-ENL and MLL-AF9 in the humanized model (Barabe et al., 2007; Wei et al., 2008). Thus, future studies using murine or humanized MLL models should provide further insights into this critical issue.

Targeting the Untargetable

MLL leukemias, in particular patients with MLL-AF4, respond poorly to current cancer therapeutics; therefore, development of more effective and specific regimens is urgently needed. While directly targeting an oncogenic transcription factor has proven to be difficult, emerging evidence indicates that MLL fusions activate downstream target genes via specific recruitment of histone modification enzymes, which can be potential therapeutic targets. DOT1L and PRMT1, two distinctive histone modification enzymes mediating H3K79 and H4R3 methylation, respectively, have been independently linked to MLL-mediated pathogenesis (Cheung et al., 2007; Okada et al., 2005). Direct fusion of PRMT1 or part of DOT1L to truncated MLL is sufficient to transform primary hematopoietic cells, and the transformations are dependent on the catalytic activity of these enzymes. Moreover, downregulation of PRMT1 expression can specifically suppress transformation mediated by certain MLL fusions but not E2A-PBX, revealing the potential of targeting specific epigenetic modifying enzymes for cancer therapeutics (Cheung et al., 2007). The current study by Armstrong and colleagues (Krivtsov et al., 2008) demonstrating the significance of DOT1L and the associated histone mark for disease classification and activation of critical downstream targets further endorses this idea (Figure 1). However, it is notable that DOT1L recruitment is ubiquitously associated with gene transcription and that a critical level of DOT1L is required for general cell survival (Okada et al., 2005). Reduction of mDOT1L by over 70% results in growth arrest and apoptosis in both hematopoietic and nonhematopoietic cells. Knocking down mDOT1L expression by just 50% inhibits primary transformed cells regardless of the interaction between DOT1L and the MLL fusions. Thus, the apparently

universal and essential function of DOT1L in multiple cell types may limit its therapeutic potential unless a specific therapeutic window can be identified to distinguish normal versus leukemic cells. However, the emerging identification of the epigenetic networks and modifying enzymes deregulated by MLL fusions will no doubt continue to provide novel insights and therapeutic avenues to target these classically nondruggable oncoproteins (Figure 1).

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Inflammation Joins the "Niche"

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Bone marrow-derived cells have an important role in tumor metastasis and have been reported to "prime" distant tissues for tumor engraftment, although the mechanisms for their recruitment have remained unclear. A new study by Hiratsuka et al. describes an inflammatory signaling pathway that mediates the chemoattraction of myeloid and tumor cells to organ-specific metastatic sites.

Tumor metastasis is responsible for approximately 90% of all cancer-related deaths. It has now been well established that in order to metastasize from the primary tumor, cancer cells need to acquire additional properties that enable invasion of the extracellular matrix, intravasation, travel through blood vessels, migration to and invasion at the secondary site, and formation of metastatic nodules (Nguyen and Massague, 2007). Importantly, tumor cells are not the only participants in this complex process, as tumor-associated cells such as macrophages and bone marrow-derived progenitors have also been implicated in tumor progression and metastasis. There appears to be a collaborative role for these nonmalignant cells in enhancing metastasis, as they "precondition" the microenvironment in potential sites of metastasis, promoting tumor invasion (as reviewed in Wels et al., 2008).

The elegant early studies by Paget and Ewing first described the "seed and soil" hypothesis, suggesting that microenvironmental factors together with mechanical forces of the circulation were both important determinants of site-specific metastatic spread. Furthermore, metastatic progression is considered an evolutionary process that requires acquisition of additional genetic alterations, conferring a selective advantage to unique clones within the tumor cell population and allowing those clones to metastasize (Nguyen and Massague, 2007). Recently, studies have shown that factors derived from the primary tumor mediate the establishment of specific microenvironments in distant organs that are sites of future metastasis, the so-called "premetastatic niche." But how is it possible that alterations in the microenvironment of distant organs can occur even before the first metastatic tumor cell has arrived? The molecular pathways that underlie premetastatic niche formation are the focus of intensive ongoing study to elucidate the signaling paradigms that define future secondary tumor foci.

Over the last several years, a crucial role for bone marrow-derived cells (BMDCs) in priming distant tissues for tumor metastasis has been uncovered (Hiratsuka et al., 2006; Kaplan et al., 2005). It has been shown that cells of the hematopoietic lineage populate distant organ tissues prior to the arrival of the tumor