Turning on a dimer: New insights into MLL chimeras

In this issue of *Cancer Cell*, So et al. (2003) demonstrate a novel mechanism for the oncogenic activity of MLL chimeric proteins. By providing coiled-coil or other dimerization domains, the cytoplasmic partners of MLL fusion proteins donate a platform for MLL homodimerization, allowing recruitment of accessory factors needed to activate the critical downstream targets, including selected subsets of the major *HOX* genes.

There are many compelling reasons to study mixed lineage leukemia (*MLL*) fusion genes in human leukemia. From a purely clinical perspective, the expression of MLL fusion proteins by leukemic cells almost always confers a worse prognosis. Such alterations are found in 80% of acute lymphoblastic leukemias (ALLs) and acute myeloid leukemias (AMLs) that arise in infants, almost all cases of secondary AML that arise after treatment with topoisomerase II inhibitors, and at least 10% of de novo ALLs and AMLs in children and adults. Of considerable fundamental importance is the opportunity to elucidate the signaling pathways usurped by chimeric MLL oncoproteins, including those initiated by the direct downstream targets of MLL, such as the major *HOX* genes, which encode highly conserved transcription factors with critical roles in both embryonic and hematopoietic differentiation.

Identified by cloning human chromosome band 11q23 breakpoints, *MLL* was shown to be the mammalian counterpart of *trithorax*, the *Drosophila* gene that encodes a nuclear regulatory protein required for the maintenance of specific spatial patterns of *HOM-C* gene expression induced during embryogenesis (Schumacher and Magnuson, 1997). Mammalian cells contain 39 different major *HOX* genes, grouped in clusters (*HOXA* to *HOXD*) on four separate chromosomes, which share extensive homology with the *HOM-C* genes of *Drosophila* and play major roles in axial morphogenesis and patterning (Maconochie et al., 1996). These relationships predict that normal MLL, and possibly the leukemogenic fusion proteins generated from it by chromosomal translocation, might function as epigenetic regulators of the corresponding *HOX* genes in humans and other mammals. This hypothesis was shown to be correct in mice, in which heterozygous germline inactivation of *Mll* resulted in homeotic transformations of the axial skeleton associated with abnormalities in the maintenance, but not the initiation, of *Hoxa7* and *Hoxc9* gene expression (Yu et al., 1995). Moreover, *HOX* genes were shown to have highly specific patterns of expression in subsets of murine and human hematopoietic stem and progenitor cells (Look, 1997; Sauvageau et al., 1994), while multiple lines of evidence supported a major role of dysregulated *HOX* gene expression in leukemogenesis (reviewed in Look, 1997). For example, high levels of *HOX* gene expression are a major component of the gene expression profiles that distinguish early B-lineage leukemias with *MLL* fusion genes from those transformed by other mechanisms (Armstrong et al., 2002; Yeoh et al., 2002). Also, comparative analysis of the gene expression profiles of T- and early B-lineage leukemias implicated upregulation of a subset of major *HOX* genes—*HOXA9*, *HOXA10*, and *HOXC6*—and the *MEIS1 HOX* coregulator as the central distinguishing feature of leukemic transformation by MLL oncoproteins (Ferrando et al., 2003). These findings raise a critical question concerning the oncogenicity of MLL: do chimeric MLL oncoproteins act on target genes through the same mechanisms exploited by wild-type MLL or have they evolved unique strategies?

Progress in dissecting normal *HOX* gene regulation has provided valuable clues to the requirements for leukemic transformation by MLL fusion proteins. Since *HOX* gene expression must not only be maintained in specific subsets of $CD34^+$ hematopoietic stem and progenitor cells but also must be downregulated as lymphoid and myeloid cells mature in the bone marrow (Sauvageau et al., 1994), any mechanism of effective gene regulation must accommodate both criteria. This issue has been partially addressed for wild-type Mll by showing direct binding of the normal Mll protein, as a component of a large multiprotein supercomplex, to the *Hoxa9* and *Hoxc8* regulatory regions in chromatin immunoprecipitation assays and by the demonstration that the SET domain of Mll is a histone H3 (lysine 4)-specific methyltransferase whose activity is associated with *Hox* gene activation (Milne et al., 2002; Nakamura et al., 2002). Recent studies have further demonstrated that wild-type MLL is processed into two fragments—a carboxy-terminal fragment with strong transcriptional activation properties and an amino-terminal fragment with transcriptional repression activity (Hsieh et al., 2003; Yokoyama et al., 2002) (Figure 1). The C-terminal fragment contains the SET domain and the TAD domain, which possesses transcriptional activation capacity. The N-terminal fragment comprises a series of AT-hook motifs that bind DNA; the SNL1 and SNL2 regions, which mediate speckled subnuclear localization; a CXXC region with homology to a DNA methyltransferase that possesses transcriptional repressor activity; and the PHD zinc finger domain, which is bisected by MLL chromosomal translocations. Thus posttranslational cleavage of MLL may provide an on-off mechanism that would resolve the seemingly contradictory repressor and activator activities of MLL.

Recently, it was shown that *Hoxa9*-deficient bone marrow cells could not be transformed in vivo by transduction with a retroviral *MLL* fusion gene construct highly active in the leukemic transformation of normal mouse bone marrow cells, providing the first direct evidence that activation of *Hoxa9* may be required for leukemic transformation mediated by MLL fusion proteins (Ayton and Cleary, 2003). A similar genetic approach was used to show that Hoxa7 is also required for full in vitro oncogenicity of the MLL fusion protein. The N-terminal fragment of MLL, which is consistently involved in fusions with varied protein partners, lacks a SET domain and therefore could not be expected to facilitate transcription through the normal route of histone methylation (Milne et al., 2002). This suggests that the mechanism(s) underlying aberrant regulation of *HOXA9* and other downstream targets of MLL fusion proteins may differ radically from that of normal MLL (see Figure 1).

So and coworkers, in this issue of *Cancer Cell*, provide an intriguing piece of the MLL oncoprotein/*HOX* gene puz-

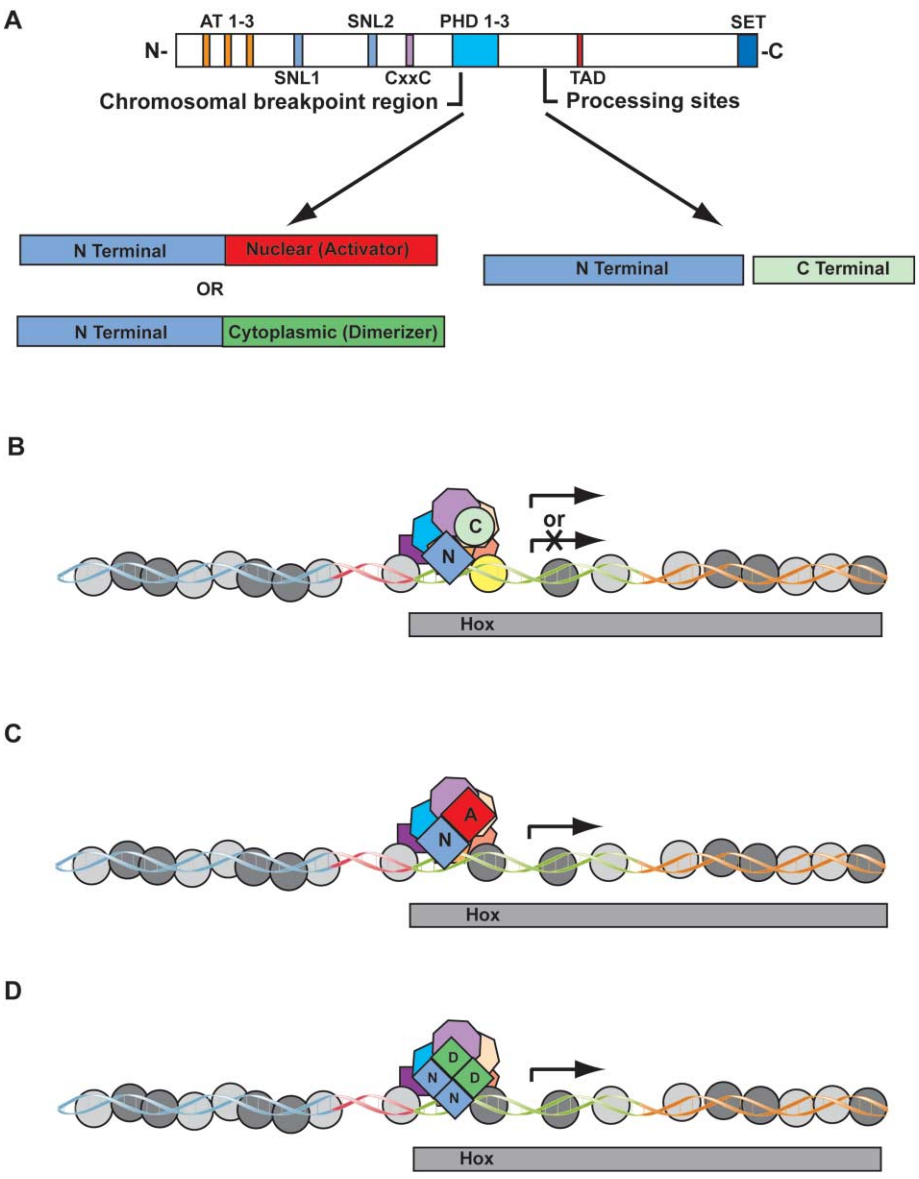

Figure 1. Different mechanisms of *HOX* gene regulation by MLL proteins

A: Wild-type MLL is usually cleaved into N-terminal and C-terminal moieties, but N-terminal residues can also participate in leukemogenic fusions with either nuclear (activator) or cytoplasmic (dimerizer) proteins. Putative functional domains are shown in color and designated as follows: AT 1-3, AT-hook DNA binding motifs 1, 2, and 3; SNL1 and 2, speckled nuclear localization signals 1 and 2; CxxC, cysteine-rich motif homologous to DNA methyltransferase and MBDI; PHD 1-3, PHD fingers 1, 2, and 3; TAD, transactivation domain; SET, histone methyltransferase domain.

B: In gene regulation by wild-type MLL, the N-terminal and C-terminal moieties cooperate in a large multiprotein supercomplex that can remodel, acetylate, deacetylate, and methylate histones (gray circles, unmethylated histone; yellow, methylated) to regulate *HOX* gene transcription. Wild-type MLL is required for the maintenance of *HOX* gene expression by hematopoietic stem and progenitor cells, but mechanisms must also exist that extinguish *HOX* gene expression as myeloid and lymphoid cells differentiate.

C: Some chimeric MLL proteins with nuclear fusion partners act as monomers to constitutively activate *HOX* gene expression in leukemic stem cells, while others with cytoplasmic partners (**D**) rely on forced homodimerization to constitutively activate *HOX* gene transcription.

zle, showing that simple dimerization of MLL by a cytoplasmic fusion partner (either GAS7 or AF1p) activates its transcriptional and oncogenic properties. These include upregulation of the murine *Hoxa7*, *a9*, and *a10* genes, as well as the Hox cofactor gene *Meis1*, which otherwise are repressed in differentiating myeloid progenitors. This mechanism is especially relevant in light of the remarkable array of nuclear and cytoplasmic proteins (more than 40 by last count) that can fuse with MLL. Comparison of these results with *HOX* gene expression profiles produced by MLL-ENL, which displays intrinsic transcriptional effector activity, indicated similar effects for both classes of MLL fusion proteins, suggesting that the *Hox* gene specificity of MLL fusion proteins may not be significantly affected by the lack of classical transcriptional effector domains in cytoplasmic versus nuclear fusion partners.

Oligomerization of MLL through the coiled-coil domains of the cytoplasmic GAS7 and AF1p proteins helps to explain how widely divergent fusion partners might achieve the same end result, leukemic transformation, but leaves unresolved the critical issue of a mechanism for dysregulation of *HOX* gene expression. Wild-type MLL encodes a histone methytransferase that has been reported to assemble into a supercomplex (Nakamura et al., 2002) containing several chromatin-modifying components that can remodel, acetylate, deacetylate, and methylate nucleosomal histones. MLL normally maintains *HOX* gene transcription in part by targeting SET domain methyltransferase activity to *HOX* gene promoters (Milne et al., 2002), but this domain as well as the TAD transcriptional activation domain are lost by leukemogenic MLL fusion proteins. Thus, So and coauthors suggest that forced dimerization of MLL creates a transcriptional activator complex capable of stimulating *HOX* expression in the absence of histone methylation. They further propose that MLL dimerization may lead to the inappropriate recruitment of accessory factors required for sustained expression of *HOX* genes. Interestingly, this model may also account for the oncogenicity of a class of transforming "self-fusion" MLL proteins, which arise through an intrachromosomal tandem duplication of N-terminal *MLL* sequences, effectively producing a tethered homodimer of this region fused to the remainder of the MLL coding sequences (Schichman et al., 1994).

The elegant demonstration of a novel mechanism whereby MLL fusion proteins could engage *HOX* genes in malignant transformation strengthens

the argument for selected *HOX* genes being preferred targets of this class of leukemogenic transcription factors. Once the downstream targets of the vertebrate HOX proteins are identified, both in embryologic development and in leukemogenesis, it should be possible to expand on current models of *HOX* gene involvement in the human leukemias, hopefully yielding new targets for therapeutic intervention.

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Selected reading

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MT1-MMP: A collagenase essential for tumor cell invasive growth

The manuscript discussed in this preview describes that reconstituted three-dimensional extracellular matrices such as fibrillar collagen and fibrin exert stringent territorial growth control on cells. The authors show that tumor cells are able to escape the matrix-enforced growth control effect (entrapment) by pericellular proteolysis mediated by MT1-MMP, a membrane bound matrix metalloproteinase capable of directly cleaving both type I collagen and fibrin but not by other, soluble matrix metalloprotinases. These data convincingly demonstrate one way that tumor cells orchestrate proteolysis to invade surrounding tissues.

Tumor cell breach of physiological barriers defines the point in neoplastic growth when medical intervention becomes infinitely harder. The breach of such barriers has often been associated with expression of one of more matrix metalloproteinases, a family of Zn-dependent endopeptidases that collectively are capable of cleaving virtually all extracellular matrix substrates. In the July 11 issue of *Cell*, Hotary et al. (2003) demonstrate that cancer cells rendered capable of expressing a single proteinase, namely the membrane bound matrix metalloproteinase-1 (MT1-MMP) (Sato et al., 1994), acquire potent collagenolytic activity that enables cell proliferation in a three-dimensional collagen matrix or fibrin matrix and moreover enhances subcutaneous growth of tumor cells in nude mice. Remarkably, a similar effect is not conferred by any of seven soluble matrix metalloproteinases, including three "classical" collagen-cleaving proteinases (MMP-1, MMP-2, and MMP-13) (Brinckerhoff and Matrisian, 2002; Egeblad and Werb, 2002). Equally surprising, the growth-promoting effect of MT1-MMP was lost on planar, 2D surfaces or under circumstances where the 3D matrix is not degradable. The significant difference between behavior in 2D and 3D mirrors a previous observation by Cukierman et al. (2001) on cell-matrix adhesive properties.

Taken together, the observations in the Hotary et al. paper have several important implications. They support the notion that pericellular interstitial collagenolytic activity rests with a single molecule—namely the tethered transmembrane collagen-cleaving member of the MMP family (Brinckerhoff and Matrisian, 2002; Egeblad and Werb, 2002) that alone is capable of conferring collagen-degrading abilities to otherwise incompetent cells. Although the predominant paradigm for the role of MT1-MMP in matrix degradation has been to act as an activator of downstream-secreted MMPs, Hotary et al. provide direct evidence for an intrinsic collagenase activity of MT1-MMP. Whereas the lack of impairment of collagen degradation in several MMP knockout models could in principle be ascribed to the obvious redundancy of the system, MT1-MMP depletion alone is sufficient to generate a "collagen-indigestion" phenotype in the mouse (Holmbeck et al., 1999). Considering that MT1-MMP alone can provide a tumor cell with invasiveness, whereas secreted MMPs cannot, the question arises whether additional tethered MMPs can do it.

Hotary et al. moreover demonstrate