Structural basis of multimer-mediated mayhem

Oligomerization of AML1-ETO contributes to leukemogenesis through obscure mechanisms. In this issue of *Cancer Cell*, Bushweller and colleagues show the crystal structure of the ETO NHR2 domain to be a tetramer. Tetramer formation is important for maturation arrest and self-renewal, and gene expression is altered in the absence of self-association. Loss of oligomer formation disrupts interactions between AML1-ETO and members of the ETO corepressor family, but not other corepressor molecules posited to be important for leukemogenesis. The findings clarify the role of oligomer formation in AML1-ETO function and suggest a possible therapeutic strategy of targeting ETO-corepressor interactions.

The t(8;21) chromosomal translocation is a common genetic event in myeloid leukemias and is associated with a relatively favorable prognosis. The resultant AML1-ETO fusion protein is thought to interfere with normal AML1 (RUNX1) function, since the *AML1-ETO* knockin mouse phenocopies *AML1* knockout mice (reviewed in Hess and Hug, 2004). The mechanism of transformation has been studied extensively, and two key findings have emerged: AML1-ETO requires second "hits" to cause leukemia, and conserved domains within ETO are responsible for derangement of normal AML1 transcriptional activity. These domains, known as Nervy homology regions (NHRs) based upon similarity with *Drosophila* homolog Nervy, confer important properties to the AML1-ETO fusion, including corepressor recruitment and self-association potential.

Self-association is crucial to the pathogenesis of many fusion oncoproteins, and may be an attractive target for anticancer therapy (So and Cleary, 2004). How does oligomerization result in neoplasia? The mechanisms are varied. The most straightforward examples are tyrosine kinase fusions such as the BCR-ABL fusion found in chronic myeloid leukemia: dimerization of the ABL kinase results in autophosphorylation, constitutive kinase activity, and resultant downstream signaling dysregulation. Among transcription factor oncoproteins, the mechanisms are less clear—but in general invoke altered DNA binding site occupancy and changes in protein partner affinity. For example, our group has reported that dimerization of MLL enhances DNA binding affinity and increases expression of target genes (Martin et al., 2003). However, there is also substantial evidence that self-association is insufficient for transformation: recent work in this journal shows that enforced dimerization of RARα fails to recapitulate the differentiation block and immortalization seen with PML-RARα expression (Licht, 2006). In many cases, the additional functions conferred by the fusion partner are spatially inseparable from the dimerization domain itself: this overlap confounds mutation analysis of oncoproteins.

Oligomerization of AML1-ETO has been documented by several groups (Zhang et al., 2001, and others), and the activity has been mapped to NHR2. Self-association of AML1-ETO is not *sufficient* for the leukemic phenotype, since truncations retaining NHR2 do not exhibit full activity (Lutterbach et al., 1998). But is oligomerization *necessary*? Although deletion of NHR2 is detrimental to AML1-ETO function, interpretation of this result is confounded by the multifunctional nature of the domain—it recruits mSin3A, for example, and binds histone deacetylase 2 (Amann et al., 2001). In this issue of *Cancer Cell*, Bushweller and colleagues use rigorous structural analysis to overcome this limitation (Liu et al., 2006). They report that the NHR2 domain forms homotetramers. By using crystal structure data to design oligomer-incompetent mutants, the investigators elucidate the precise functional contribution of AML1-ETO tetramer formation.

AML1-ETO is a tetramer

Bushweller and colleagues began by solving the crystal structure of the ETO NHR2 domain, which is responsible for self-association of AML1-ETO. Analysis revealed kinked α-helical monomers paired together in antiparallel dimers, each of which is in turn bundled with another dimer. The resultant tetramer forms a left-handed supercoil that buries the hydrophobic surface of each amphipathic monomer helix; the complex is further stabilized by intermolecular salt bridges. The overall structure contains several familiar motifs—including a general similarity to the supercoiled heterotrimeric SNARE complex, as well as specific intermolecular contacts that are seen in leucine zippers. However, Bushweller and colleagues are the first to describe an interaction scheme consisting of a central tetrameric region of ten helical turns flanked by 5-turn C-terminal dimer tails at each end. Mass estimation by sedimentation velocity confirmed that, even in solution, the NHR2 domain indeed exists as a tetramer.

Effect of NHR2 mutation on phenotype

Solving the crystal structure of the NHR2 domain allowed Bushweller and colleagues to design mutations that disrupt either AML1-ETO oligomerization or interaction with mSin3A. These precise, data-driven substitutions represent an elegant improvement upon traditional slice-and-dice modalities such as domain deletion and random point mutagenesis.

By comparing these mutants to wild-type AML1-ETO and the NHR2-deleted fusion protein, Bushweller and colleagues were able to definitely attribute various aspects of the leukemogenic phenotype to tetramer formation. The investigators addressed the two fundamental components of neoplastic transformation: differentiation arrest and clonogenic potential. The NHR2 domain has previously been implicated in maturation arrest: whereas AML1-ETO expression blocks mouse bone marrow cell differentiation, the NHR2-deleted mutant impairs this capability. Bushweller and colleagues found that tetramer-incompetent full-length AML1-ETO behaved like the NHR2 deletion mutant, while another tetramer-weak mutant showed an intermediate phenotype. This suggests that self-association contributes to the differentiation arrest that characterizes leukemia.

Self-renewal of AML1-ETO-expressing cells is also well established (Hug et al., 2002). Although Hug et al. (2002) have reported that NHR2 is dispensable for this function, the current work by Bushweller and colleagues suggests otherwise: they found that the NHR2 deletion as well as the complete and partial tetramer-incompetent mutants were unable to sustain clonogenic activity. Interestingly, the mSin3A binding mutant did confer self-renewal as efficiently as wild-type AML1-ETO.

Pathways affected by tetramer formation

The investigators then focused on the precise functional deficiencies of tetramer-incompetent variants. As AML1-ETO acts as a transcriptional activator or repressor in different contexts, it is not surprising that Bushweller and colleagues found nonuniform changes in gene expression when comparing the wild-type protein to the tetramer-incompetent mutant. For genes such as those coding for M-CSF receptor and neutrophil elastase, the derepression seen with the mutant correlates well with loss of hematopoietic differentiation block. Similarly, blunted activation of c-*Fos* by the mutant may contribute to its clonogenic impotence in self-renewal assays. The findings suggest that oligomerization informs transcriptional regulation by AML1-ETO.

Previous work has provided a long list of interaction partners for AML1-ETO, and some investigators have speculated that multimerization of AML1-ETO modulates the affinity of these factors (Zhang et al., 2001; Hug and Lazar, 2004). By precisely disrupting self-association, Bushweller and colleagues were able to assess the true contribution of tetramer formation to partner binding. Not surprisingly, interactions with wild-type ETO and ETO family members are lost when self-association of the ETO moiety of AML1-ETO is lost. This finding is in fact a confirmation that oligomer formation is impaired in the mutant. However, it also highlights an alternative interpretation of the phenotype experiments: perhaps loss of interaction between AML1-ETO and wild-type ETO (or another ETO family member) mediates the loss of maturation arrest and/or self-renewal.

With the exception of ETO family members, the tetramer-incompetent mutant retains affinity for all interaction partners tested, including histone deacetylase enzymes 1–3, the corepressors N-CoR, SMRT, and mSin3A, and several other known interactors. In contrast, the NHR2 deletion mutant demonstrates impaired interaction with mSin3A and HDAC2—again verifying the precise disruption of tetramer formation in the targeted mutant. The retention of binding affinity for these cofactors by the AML1-ETO monomer is a surprise, as previous work suggested dependence upon the oligomerized state (Zhang et al., 2001) However, the mechanism may still be valid for other untested interaction partners. In addition, self-association may increase the local concentration of the tested factors by up to 4-fold—and by doing so contribute to the leukemic phenotype.

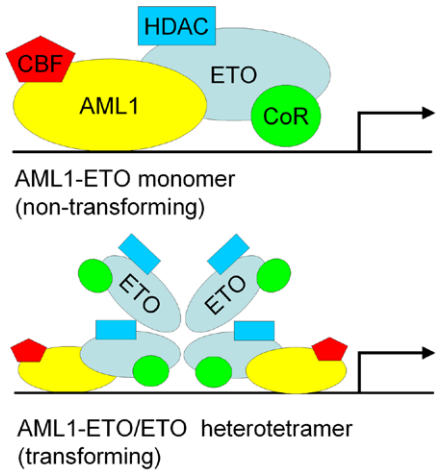

Figure 1. Functional consequences of AML1-ETO tetramer formation

The AML1-ETO monomer (top) is capable of binding to target loci and recruiting corepressors and deacetylases (N-CoR, mSin3A, SMRT, HDAC1–HDAC3) but is nontransforming. Oligomerization of AML1-ETO (bottom) allows recruitment of additional ETO family members and confers transforming capability. Other mechanisms to be defined may also contribute to the leukemogenicity of the oligomer, including altered DNA binding affinity, recruitment of additional corepressors, or increased local corepressor concentration.

While the current study by Bushweller and colleagues clarifies a fundamental point in the AML1-ETO field, it also raises many interesting questions. Does heterotetramer formation between AML1-ETO and ETO family members play a role in pathogenesis (Figure 1)? What functional properties does tetramer formation provide, other than the capability of interacting with other NHR2-containing proteins? Perhaps higher-order complex formation allows AML1-ETO and other transforming fusion oncoproteins to function at concentrations far below that of their constituent proteins. Is DNA binding modulated by oligomerization? Does tetrameric structure affect the ability of AML1 to heterodimerize with core binding factor subunit β? Are other conserved domains capable of symmetric interaction with NHR2? With at least a handful of possible subunits and four available places in each multimer, the combinatorial possibilities for AML1-ETO-containing complexes are dizzying. Finally, this study raises the possibility that disrupting oligomer formation, or more likely, ETO corepressor recruitment, might be an effective therapeutic target in this common form of leukemia.

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