inside blood

Of mouse, not of man? a surprise at the α-globin locus

Cellular and transgenic models have provided important insights into the molecular mechanisms required for mammalian globin gene expression. The homology of the human and murine α- and ζ-promoter sequences coupled with in vivo transcriptional analysis suggested that the genes were controlled in a similar manner in murine and human erythroid cells. The identification of 4 upstream erythroid-specific DNaseI hypersensitive (HS) sites in both species confirmed the overall structural similarity of the α-loci. Support for a conservation of function was provided by the demonstration that some HS sites enhance transcription significantly when linked with a globin promoter in *cis*. These studies resulted in a model in which the sequences required for developmental-specific expression reside in gene-proximal promoters, the upstream HS being required for high-level tissue-specific expression.

A key test of this model is provided by Anguita and colleagues (page 3450), in which they determine the function of a key upstream HS site at the endogenous murine α-globin locus. Previous work by this group using a chromosome 16 somatic hybrid cell line demonstrated that disruption of hHS −40, a single HS site located 40kb upstream of the human α-globin genes, resulted in complete loss of α-globin gene expression. In the present study, homologous disruption of the apparently functionally equivalent murine HS site, mHS −26, was performed with the expectation of a resultant severe α-thalassemic phenotype in animals homozygous for the deletion. In contrast to the human studies, homozygotes have relatively normal erythropoiesis except when subjected to anemia-inducing phenylhydrazine stress. This result is reminiscent of similar studies deleting individual HS sites in the murine β-globin locus control region (LCR) (Bender et al, Mol Cell. 2000;5:387-393).

Several potential explanations for the species-specific difference in outcome can be entertained. These include the unique telomeric location of the human locus when compared to the interstitial position of its murine ortholog, a modest divergence in factor binding sites between the mHS −26 and hHS −40 regions, and/or the confirmed differences in enhancer action of hHS −40 and mHS −26, as measured in murine erythroleukemia cells as reported here. An equally attractive hypothesis, linking many of these observations, is that 2 or more HS sites may be required for appropriate murine α-globin gene expression. In contrast, hHS −40 may be sufficient at the human locus. Further experimentation at the endogenous loci will be required to distinguish these possibilities. Indeed, it is likely that further Darwinian surprises are in store that will remind us that murine and human globin biology may not be one and the same.

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Phages that display P-selectin antagonism

Selectins mediate adhesion events among leukocytes, platelets, and endothelial cells. Within the selectin family, P-selectin plays a predominant role in the recruitment of polymorphonuclear neutrophils into inflammatory sites and, thus, represents an important target for future therapies against various inflammatory diseases. Despite intense research efforts over the past 20 years, effective selectin inhibitors have not yet become available to clinicians. Since all 3 selectins bind sialylated and fucosylated oligosaccharides (for example, sialyl Lewis X), much of the work has focused on the generation of specific glycoconjugates that generally display relatively low binding affinities when presented as mono- or oligovalent ligands. In its natural environment, the chief P-selectin glycoprotein ligand (PSGL-1) indeed requires both the specific carbohydrate decorations and sulfated N-terminal tyrosine residues to bind P-selectin with high affinity (nanomolar range).

Molenaar and colleagues (page 3570) used phage-display libraries to isolate peptides containing the consensus sequence EWVDV, which can specifically inhibit human P-selectin but not mouse P-selectin or human E- or L-selectins. The binding affinity of an EWVDV-containing peptide for P-selectin was greatly enhanced (200-fold) when presented as a tetramer. Importantly, Molenaar et al also show that nanomolar concentrations of the tetrameric peptide can efficiently inhibit the adhesion of HL60 cells to P-selectin in static assays and increase rolling velocities of HL60 cells in a flow system. How do such small peptides do it? Because the EWVDV-containing peptide can inhibit the binding of a carbohydrate selectin ligand (sulfated Lewis A), the authors speculate that EWVDV may interact in close proximity of the carbohydrate recognition domain of P-selectin. The fact that EWVDV binding to P-selectin is only partially Ca^{++} dependent suggests that the peptide may also interact outside the carbohydrate-binding pocket whose binding is strictly Ca^{++} dependent. Since the consensus sequence contains 2 acidic residues (glutamic acid and aspartic acid), its overall negative charge may also promote interactions with the region of positive electrostatic potential previously shown to mediate high-affinity interactions with the sulfotyrosines of PSGL-1 (Somers et al, Cell. 2000;103: 467-479). This feature might also explain the dramatically enhanced avidity of multimeric peptides. Beyond their physicochemical properties, the real litmus test for the peptides will be to determine their capacity to inhibit leukocyte adhesion on activated endothelium in vivo. This remarkable study, however, provides a critical first step in the generation of powerful selectin antagonists that have profound clinical relevance.

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