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HOX deregulation in acute myeloid leukemia

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The deregulation of homeobox (HOX) genes in acute myeloid leukemia (AML) and the potential for these master regulators to perturb normal hematopoiesis is well established. To date, overexpression of HOX genes in AML has been attributed to specific chromosomal aberrations and abnormalities involving mixed-lineage leukemia (MLL), an upstream regulator of HOX genes. The finding reported in this issue of the JCI by Scholl et al. that caudal-type homeobox transcription factor 2 (CDX2), which is capable of affecting HOX gene expression during embryogenesis, is overexpressed in 90% of patients with AML and induces a transplantable AML in murine models provides an alternative mechanism for HOX-induced leukemogenesis and yields important insights into the hierarchy of HOX gene regulation in AML (see the related article beginning on page 1037).

Acute myeloid leukemia (AML) is a heterogeneous disease in which hematopoietic progenitor cells acquire genetic lesions that lead to a block in differentiation, increased self-renewal, and unregulated proliferation. The emergence of leukemic blasts appears to require at least two major genetic “hits,” involving perturbations in growth factor signaling pathways and hematopoietic differentiation programs (1).

Among the receptor tyrosine kinases (RTKs), fms-like tyrosine kinase 3 (FLT3), which plays important roles in hematopoietic progenitor cell survival and

proliferation, is overexpressed in a significant proportion of AMLs, and mutations resulting in the constitutive activation of FLT3 occur in approximately 33% of patients (2). Mutations leading to the constitutive activation of a related RTK, c-KIT, and of signaling intermediates such as RAS, are also frequently described in AML (3). The dysregulation of associated signaling pathways (e.g., Ras/MAPK, PI3K/AKT, and JAK/STAT) is thought to result in growth factor-independent proliferation and clonal expansion of hematopoietic progenitors.

The second hit targets transcription factors capable of disrupting hematopoietic cell differentiation. This may occur following the dysregulation of specific gene regulators as a result of gene amplification (e.g., *v-myc myelocytomatosis viral oncogene homolog* [MYC], *mixed-lineage leukemia* [MLL], genes at the chromosome 11q23 locus) (4); point mutations in transcriptional regulators (e.g., *CCAAT/enhancer-binding protein* [C/EBP], *runt-related transcription factor 1* [RUNX1]); and chromosomal transloca-

tions resulting in the fusion of *promyelocytic leukemia* (PML) and the *retinoic acid receptor α* (RARA) to yield the PML-RARA chimeric protein or fusion of *RUNX1* and *runt-related transcription factor 1, translocated to 1* (RUNX1T1), which gives rise to the RUNX1-RUNX1T1 fusion protein (1). It is becoming increasingly clear that one set of genes commonly affected by these chimerical and mutated transcriptional regulators are the *homeobox* (HOX) genes. Accordingly, overexpression of homeobox master transcription factors, which fulfill critical roles in embryonic development, organogenesis, and normal hematopoietic differentiation, is a common feature of AML (5).

HOX genes: from hematopoiesis to leukemia

In mammals, HOX genes are located in two main clusters, the primordial cluster and the ParaHox cluster, which are thought to originate from the duplication of a hypothetical ProtoHox cluster of four genes early in evolution (6). The primordial HOX cluster consists of 13 paralogous groups of genes that exist as distinct, unlinked complexes on human chromosomes 7p15 (HOXA), 17q21 (HOXB), 12q13 (HOXC), and 2q31 (HOXD), and the cluster is organized such that during embryonic development, the order of expression along the anterior-posterior embryonic axis (3' to 5') is colinear with the alignment of genes on the chromosome (Figure 1). During hematopoiesis, HOX genes are expressed in lineage- and stage-specific combinations; however, cell commitment to myeloid or erythroid lineages is accom-

Nonstandard abbreviations used: AML, acute myeloid leukemia; ATRA, all-trans retinoic acid; CDX2, caudal-type homeobox transcription factor 2; ETV6, ets variant gene 6; FLT3, fms-like tyrosine kinase 3; HOX, homeobox; MEIS1, myeloid ecotropic viral integration site 1; MLL, mixed-lineage leukemia; PML, promyelocytic leukemia; RARα, retinoic acid receptor α; RTK, receptor tyrosine kinase; RUNX1, runt-related transcription factor 1.

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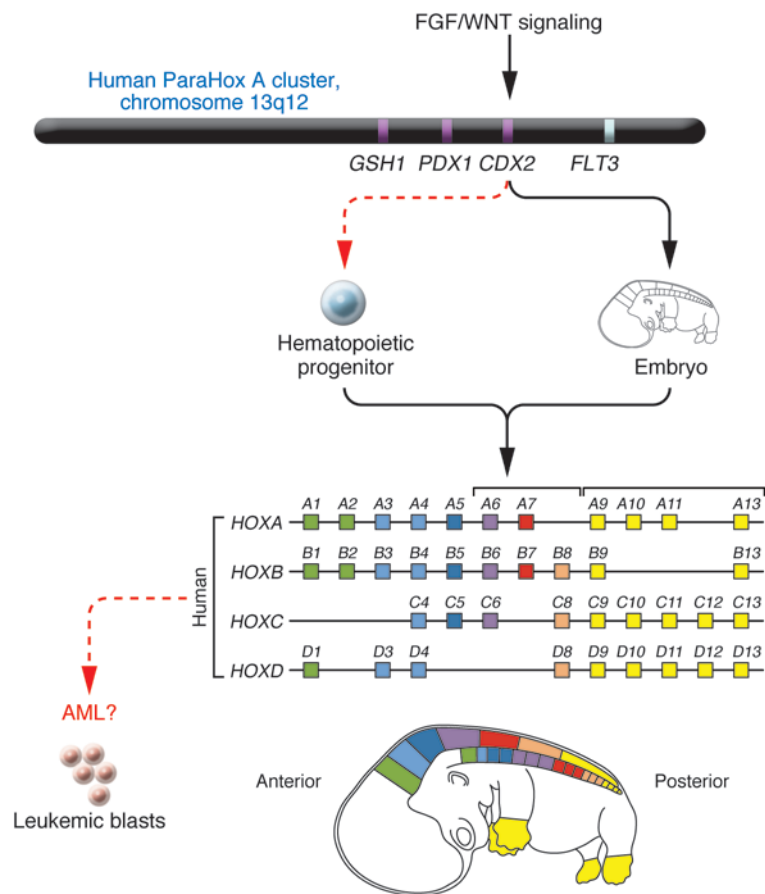


Figure 1

A model for CDX2-mediated leukemogenesis. CDX2 is expressed in the posterior primitive streak during embryogenesis, where it directs anteroposterior axial development and elongation by regulating HOX gene expression. A study in this issue of the *JCI* (12) reports that monoallelic expression of CDX2 is observed in 90% of patients with AML and may perturb hematopoiesis by affecting HOX gene expression (red dashed arrows indicate ectopic expression; black arrows indicate normal expression in embryonic development). GSH1, *GS homeobox 1*; PDX1, *pancreatic and duodenal homeobox 1*. Figure modified with permission from *Molecular Genetics and Metabolism* (21).

panied by global downregulation of HOX gene expression (7).

HOX genes may be dysregulated in AML by several different mechanisms. First, specific HOX genes can be disrupted via chromosomal translocation. Specifically, HOXA9 and HOXD13 are dysregulated through the t(7;11) and t(2;11) translocations, respectively (8), in both cases creating fusion proteins between the HOX protein and the nucleoporin 98 kDa (NUP98) nuclear protein. Overexpression of HOXA6, HOXA7, HOXA9, and the HOX cofactor myeloid ecotropic viral integration site 1 (MEIS1) has also been correlated with chromosome 11q23 abnormalities involving the MLL protein, which regulates the expression of HOX genes (9). A less frequently observed translocation in AML, t(8;16)(p11;p13), which results in the overexpression of the MYST3-CREBBP fusion protein, is also associated with overexpression of HOXA9, HOXB9, HOXA10, and MEIS1 (10). In addition, the expression of HOXC4 was shown to be upregulated in the NB4 PML-RAR α cell line following all-trans retinoic acid-induced (ATRA-

induced) differentiation as well as in bone marrow from acute PML patients during ATRA treatment, strengthening the theory that dysregulated HOX expression is a signature feature of AML (11).

CDX2 overexpression: a unifying theme in AML?

In keeping with the theme of dysregulated HOX gene expression in AML, in this issue of the *JCI* Scholl et al. (12) demonstrate that caudal-type homeobox transcription factor 2 (CDX2), a homeobox gene involved in anteroposterior axis definition and intestinal epithelial cell differentiation (13), but strikingly absent in hematopoietic progenitors, is overexpressed in 90% of patients with AML. The leukemogenic potential of CDX2 overexpression was first described by Chase et al. (14) following the identification of a novel chromosomal rearrangement, t(12;13)(p13;q12), in a patient with AML, which yielded the ets variant gene 6-CDX2 (ETV6-CDX2) fusion protein in addition to full-length CDX2. Significantly, transduction of murine hematopoietic progenitors with CDX2, but not the

ETV6-CDX2 fusion protein, resulted in a transplantable and fatal AML. Although ETV6-CDX2 induced myeloproliferation, it did not induce leukemia or accelerate CDX2-mediated leukemogenesis (15), suggesting that the primary oncogenic affect of this translocation is the ectopic expression of CDX2 in the hematopoietic compartment (Figure 1).

CDX2 belongs to the ParaHox A cluster, which consists of a three-gene complex including *GS homeobox 1* (GSH1), *pancreatic and duodenal homeobox 1* (PDX1), and CDX2 that exists on chromosome 13q12 (6) (Figure 1). On the basis of sequence similarity, CDX is more related to the posterior group of HOX genes, which includes paralogous groups 9–13, and during embryonic development is expressed in the posterior primitive streak to regulate HOX genes and in posterior signaling pathways to direct anteroposterior axis development and elongation. Given the role of CDX2 as a regulator of HOX genes in development, it is therefore not surprising that ectopic expression of CDX2 in hematopoietic progenitors perturbs blood cell differentia-



tion programs strictly regulated by *HOX* genes. The finding by Scholl et al. (12) that overexpression of *Cdx2* in primary murine hematopoietic progenitors resulted in transplantable AML in vivo, coinciding with a three-fold upregulation in *Hoxb6* expression, is particularly intriguing given that overexpression of *HOXB6* has been documented in approximately 40% of human AMLs that do not have chromosomal translocations (16). Identifying whether *Cdx2* directly regulates *Hoxb6* and providing evidence of the specific involvement of *Hoxb6* in *Cdx2*-mediated leukemogenesis would strengthen the hypothesis that dysregulated *HOX* gene expression by *CDX2* is a major pathway contributing to leukemogenesis.

The dysregulation of *HOX* genes by members of the caudal *HOX* family may prove to be a unifying theme in AML, particularly in light of a recent study by Bansal et al. (17) that revealed dysregulated *CDX4* expression in 25% of AMLs. However, whereas *CDX2* induced rapid AML in bone marrow transplant recipients, *CDX4* induced a partially penetrant, long-latency AML, suggesting that these transcription factors regulate distinct sets of genes, including potentially different subsets of *HOX* genes. In addition to dysregulating *HOX* genes, *CDX2* overexpression may also affect signaling pathways. *Cdx1/2* mutant embryos demonstrate posterior body truncations that phenocopy loss-of-function *Wnt3a* and *Fgfr1* mutants (18), suggesting an overlap among *Cdx*, *Wnt*, and *Fgf* pathways in axial patterning. This implies that some *CDX2* target genes might include components of signaling pathways or that *CDX2* itself may be a target of cell-signaling regulators, a point highlighted by Scholl et al. (12), given that related family members *CDX1* and *CDX4* are direct targets of the *Wnt* pathway commonly activated in AML. Indeed, Scholl et al. showed that knockdown of *CDX2* slowed cellular proliferation specifically in *CDX2*-expressing AML cell lines. Taken together, these data suggest that gain-of-function mutations of *CDX2* might augment cell signaling in addition to disrupting differentiation programs. This may explain the rapid onset of AML in mice transplanted with *CDX2*-expressing hematopoietic progenitors.

Unraveling the mechanism

The molecular basis for the overexpression of *CDX2* remains elusive, and the absence of chromosomal rearrangements or mutations in the coding sequence or

proximal promoter of *CDX2* suggest that other undefined regulatory regions driving *CDX2* expression may be a target for mutation. Indeed, aberrant expression of *CDX2* in the absence of genetic abnormalities on chromosome 13 was previously documented in a case of chronic myeloid leukemia blast crisis (14). Intriguingly, microarray analysis of 50 AML patient specimens revealed that the highest levels of *HOX* expression are associated with a subset of patients with intermediate cytogenetics and elevated levels of *FLT3* mRNA and *FLT3* mutations. In the absence of mutations or amplifications, overexpression of *FLT3* was correlated with aberrant expression of multiple *HOX* genes, suggesting that deregulation of *HOX* genes may be due to aberrant growth factor signaling (19). Given the close proximity of the *FLT3* gene to the *CDX2* gene and the fact that both proteins are overexpressed in a high percentage of AMLs, it is tempting to speculate that *cis*-acting activating mutations in promoter or enhancer elements in the vicinity of these genes may contribute to the leukemic initiation and progression and may account for the link among *FLT3*, *CDX2*, and *HOX* gene overexpression in the absence of detectable cytogenetics (20). Detailed analyses of the regulatory sequences flanking *CDX2* and *FLT3* could provide insights into the role of these oncogenes in AML. It would also be important to correlate the level of *CDX2* overexpression in AML with *HOX* gene expression.

Identification of a *HOX* gene pathway disrupted in a substantial proportion of patients with AML (12) is a major clue to disease pathogenesis; however, more direct evidence of a link between *CDX2* overexpression and the dysregulation of specific *HOX* genes is needed. For example, demonstration by chromatin precipitation that *CDX2* occupies the promoters of genes such as *HOXB8* and *HOXB6* in *CDX2*-positive AML cell specimens would reinforce the importance of *CDX2* as an aberrant regulator of *HOX* genes. In addition, identifying the mechanism by which *CDX2* is overexpressed is crucial for unraveling the hierarchy of genetic pathways required for leukemia initiation and progression. While specific targeting of transcriptional regulators remains elusive, better understanding of the circuitry of the *HOX* genes in AML could present a new set of genetic targets, with potential therapeutic benefit for multiple AML subtypes.

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Hypoglycemia, functional brain failure, and brain death

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Hypoglycemia commonly causes brain fuel deprivation, resulting in functional brain failure, which can be corrected by raising plasma glucose concentrations. Rarely, profound hypoglycemia causes brain death that is not the result of fuel deprivation per se. In this issue of the JCI, Suh and colleagues use cell culture and in vivo rodent studies of glucose deprivation and marked hypoglycemia and provide evidence that hypoglycemic brain neuronal death is in fact increased by neuronal NADPH oxidase activation during glucose reperfusion (see the related article beginning on page 910). This finding suggests that, at least in the setting of profound hypoglycemia, therapeutic hyperglycemia should be avoided.

Hypoglycemia, including iatrogenic hypoglycemia in people with diabetes, causes brain fuel deprivation that initially triggers a series of physiological and behavioral defenses but if unchecked results in functional brain failure that is typically corrected after the plasma glucose concentration is raised. Rarely, profound, and at least in primates prolonged, hypoglycemia causes brain death.

Given the survival value of maintaining physiological plasma glucose concentrations, it is not surprising that mechanisms that normally very effectively prevent or rapidly correct symptomatic hypoglycemia have evolved (1). As a result, hypoglycemia is a distinctly uncommon clinical event except in people who use drugs that lower the plasma glucose concentration (2). Although there are other drugs, and several relatively uncommon conditions, that cause hypoglycemia (2), in the vast majority of instances the offending drug is an insu-

lin secretagogue or insulin used to treat diabetes mellitus (2, 3). As a result of the interplay of relative or absolute therapeutic insulin excess and compromised physiological and behavioral defenses against falling plasma glucose concentrations, hypoglycemia is the limiting factor in the glycemic management of diabetes (3). It causes recurrent morbidity in most people with type 1 diabetes mellitus (T1DM) and in many with advanced T2DM and is sometimes fatal. Furthermore, hypoglycemia, as well as prior exercise and sleep, further compromise glycemic defenses by causing hypoglycemia-associated autonomic failure and thus a vicious cycle of recurrent hypoglycemia. Finally, the barrier of hypoglycemia precludes maintenance of euglycemia over a lifetime of diabetes and thus full realization of the long-term vascular benefits of glycemic control.

Functional brain failure

Recent interest in alternative brain fuels (including lactate derived from glucose largely within the brain; refs. 4–6) notwithstanding, glucose is an obligate metabolic fuel for the brain under physiological conditions (7). Because the brain cannot synthesize glucose or store substantial amounts as glycogen in astrocytes, the brain requires a virtually continuous supply

of glucose from the circulation. Facilitated diffusion of glucose from the blood into the brain is a direct function of the arterial plasma glucose concentration. The rate of blood-to-brain glucose transport exceeds the rate of brain glucose metabolism at normal (or elevated) plasma glucose levels, but it falls and becomes limiting to brain glucose metabolism when arterial glucose concentrations fall to low levels (8). Thus, hypoglycemia causes brain fuel deprivation and, as a result, functional brain failure.

The sequence of responses to falling plasma glucose concentrations (1) is illustrated in Figure 1. Initially, declining plasma glucose levels activate defenses against hypoglycemia. Physiological defenses normally include decrements in pancreatic β cell insulin secretion as glucose levels decline within the physiological postabsorptive plasma glucose concentration range (approximately 3.9–6.1 mmol/l [70–110 mg/dl]). The glycemic threshold for decreased insulin secretion is approximately 4.5 mmol/l (81 mg/dl). Increments in pancreatic β cell glucagon and adrenomedullary epinephrine secretion (among other neuroendocrine responses) normally occur as glucose levels fall just below the physiological range (threshold equal to approximately 3.8 mmol/l [68 mg/dl]). If these defenses fail to abort the hypoglycemic episode, lower glucose levels trigger a more intense sympathoadrenal response that causes neurogenic (or autonomic) symptoms; neuroglycopenic symptoms occur at about the same glucose level (threshold equal to approximately 3.0 mmol/l [54 mg/dl]). The perception of symptoms, particularly neurogenic symptoms, prompts the behavioral defense, the ingestion of food. If all of these defenses fail, lower glucose levels cause overt functional brain failure that

Nonstandard abbreviations used: T1DM, type 1 diabetes mellitus.

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