Metabolism and the leukemic stem cell

Omar Abdel-Wahab and Ross L. Levine

Acute leukemias are clonal disorders of hematopoiesis wherein a leukemic stem cell (LSC) acquires mutations that confer the capacity for unlimited self-renewal, impaired hematopoietic differentiation, and enhanced proliferation to the leukemic clone. Many recent advances in understanding the biology of leukemia have come from studies defining specific genetic and epigenetic abnormalities in leukemic cells. Three recent articles, however, further our understanding of leukemia biology by elucidating specific abnormalities in metabolic pathways in leukemic hematopoiesis. These studies potentially converge on the concept that modulation of reactive oxygen species (ROS) abundance may influence the pathogenesis and treatment of acute myeloid leukemia (AML).

ROS influence normal hematopoietic stem cell (HSC) development

A series of landmark studies of HSC biology in *Drosophila melanogaster* and in mammalian systems revealed that ROS are critical in regulating the balance between HSC self-renewal and differentiation (Ito et al., 2006; Tothova et al., 2007; Owusu-Ansah and Banerjee, 2009; Fig. 1). The initial realization of the ability of elevated levels of ROS to limit HSC self-renewal came from the observation that mice lacking the ataxia telangiectasia mutated (Atm) gene develop early-onset bone marrow failure that correlated with increased ROS abundance (Ito et al., 2004). This impairment in HSC self-renewal resulted from ROS-induced activation of the p38 MAPK signaling pathway (Ito et al., 2006). Later, it was shown that regulation of ROS levels in the quiescent HSC compartment was mediated by the forkhead O (FoxO) family of transcription factors (Tothova et al., 2007). Mice with Mx-Cre recombinase-driven conditional loss of *FoxO1*, *FoxO3*, and *FoxO4* in the hematopoietic system exhibited a marked reduction in HSC numbers, as well as reduced functional evidence of HSCs in competitive and noncompetitive reconstitution assays. However, during the transition from HSC to myeloid progenitors, ROS levels increase in a manner independent of FoxO activity (Tothova et al., 2007). In fact, it is speculated that increased levels of ROS may even act as an intracellular trigger for HSC differentiation to myeloid lineage fates (Tothova and Gilliland, 2007). These findings from vertebrates were recently reinforced in studies in *D. melanogaster* that revealed that ROS accumulates at a specific stage of hematopoietic development to trigger myeloid progenitor differentiation from HSCs (Owusu-Ansah and Banerjee, 2009).

The results of these studies lend credence to the possibility that LSCs might be susceptible to elevations in ROS abundance, and that elevated ROS levels might even trigger differentiation of leukemic blasts. Prior reports that the naturally occurring molecule parthenolide may specifically target LSCs by inducing ROS further support this idea (Guzman et al., 2005). In addition, the observation that the serine-threonine kinase Akt is constitutively activated in the majority of primary AML samples suggests that some inhibition of FoxO function and increase in ROS levels are to be expected in AML samples relative to normal HSCs (Xu et al., 2003; Tothova and Gilliland, 2007).

Iron chelation therapy elevates ROS and induces AML differentiation

In this issue, Callens et al. report the effect of iron chelation therapy in AML cell lines and primary patient samples (Callens et al., 2010). They found that iron deprivation therapy, whether induced by iron chelators or by treatment with a transferrin receptor–specific antibody, promotes mature monocytic differentiation of leukemic cell lines, cultured leukemic blasts from patient samples, and normal HSCs from cord blood. Although iron deprivation may decrease ROS by decreasing substrates for the Fenton reaction, iron chelators also induce generation of ROS (Chaston et al., 2004). Indeed, the authors found that iron deprivation by deferasirox resulted in dose and time-dependent ROS formation. The investigators then used antioxidants to show that the production of ROS by iron chelating treatments was specifically responsible for monocytic differentiation of leukemia. Consistent with prior reports that activation of c-Jun N-terminal kinase (Jnk) is downstream of the oxidant stress response (Owusu-Ansah and Banerjee, 2009), iron deprivation–induced differentiation was dependent on Jnk activation. The authors also compared the gene expression signature induced by iron chelation in the HL60 cell line with that induced by differentiation caused by vitamin D3 treatment. Interestingly, a surprisingly high number of genes were up-regulated by both treatments. Moreover, iron chelation and vitamin D3 therapies synergized in inducing monocytic differentiation of leukemic cell lines, suggesting a possible shared mechanism of action.

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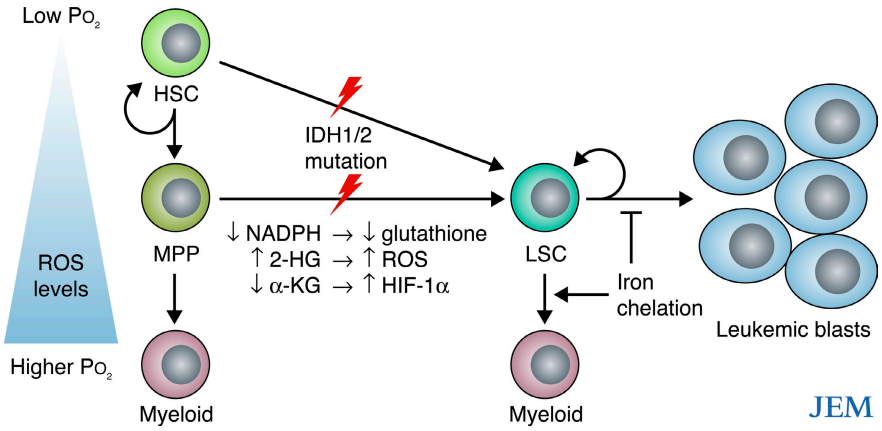

Figure 1. ROS, IDH1/2 mutations, and iron chelation therapy in normal and leukemic hematopoiesis. In normal hematopoiesis, the HSC resides in a niche characterized by a low partial pressure of oxygen (PO_2) and elevated HIF1-α. Myeloid differentiation of HSCs is triggered, in part, by increased PO_2 and increased ROS. A leukemia is thought to be a newly formed hematopoietic tissue initiated by a few LSCs. LSCs derived from either normal HSCs or more restricted multipotent progenitors (MPPs) as a result of genetic abnormalities. LSCs from AML patients may differentiate in response to a temporary increase in ROS caused by iron chelators. At the same time, mutations in the metabolic enzymes IDH1/2 observed in some AML patients may result in increased ROS and HIF-1. Mutations impair the normal enzymatic activity of IDH1/2, which is to create NAD(P)H and α-KG, and increase production of the oncometabolite 2-HG. 2-HG is thought to increase ROS concentrations. In addition, decreased abundance of α-KG results in reduced α-KG–mediated inhibition of proline hydroxylases; this facilitates increased HIF-1α stability.

The authors conclude their study with a description of a case report of an elderly patient with AML treated with the oral iron chelator deferasirox (1 g/d) and the vitamin D3 metabolite 25-hydroxycholecalciferol (4,000 IU/d). Treatment was associated with a decrease in blast counts and an increase in the production of differentiated monocytes. Although this single case report needs to be extended to formal clinical trials, it does suggest that the effects seen in cell lines are also seen in vivo. Many questions remain, including whether this is the only mechanism by which iron chelation therapy induces LSC differentiation, whether the effects of modulating ROS levels are specific to LSCs or also have negative effects on normal HSCs, and whether there are more specific agents that can be used to modulate ROS levels without the pleiotropic effects of iron chelation agents. However, these data suggest that the novel therapeutic strategy of modulating ROS levels in LSCs through the use of iron-chelating agents and/or vitamin D can induce LSC differentiation (Fig. 1).

IDH1/2 mutations result in increased ROS and hypoxia-inducible factor I (HIF-1α)

The identification of ROS levels as critical regulators of HSC quiescence and differentiation also led to the realization that the heterodimeric transcription factor HIF-1α is responsible for mediating many of the effects of hypoxia on HSCs (Adelman et al., 1999, 2000). The relative hypoxia in the HSC niche leads to increased stability of HIF-1 and up-regulation of HIF-1–mediated transcriptional targets important for maintenance of HSC quiescence, such as vascular endothelial growth factor and FoxO (Eliasson and Jönsson, 2010). Perhaps, then, it is not surprising that mutations in metabolic enzymes affecting ROS and HIF-1α levels are now being described in AML (Mardis et al., 2009; Gross et al., 2010; Ward et al., 2010).

Genome-wide sequencing studies identified somatic mutations in *isocitrate dehydrogenase 1* (*IDH1*) in central nervous system tumors and in AML patients, but not in other human malignancies (Parsons et al., 2008; Dang et al., 2009; Mardis et al., 2009; Yan et al., 2009; Zhao et al., 2009). In addition, mutations in *IDH2*, the mitochondrial homologue of *IDH1*, were identified in brain tumors expressing wild-type IDH1, but had not been identified in AML patients. *IDH1* mutations are associated with a neomorphic enzymatic activity leading to the production of the metabolite 2-hydroxyglutarate (2-HG; Dang et al., 2009). In a recent issue of *JEM*, Gross et al. (2010) screened AML samples for 2-HG production using liquid-chromatography mass-spectroscopy and found several AML tumor samples with elevated levels of 2-HG. Interestingly, although some samples with elevated 2-HG abundance harbored mutations in IDH1, a subset of samples with markedly elevated 2-HG levels expressed wild-type *IDH1*. They then demonstrated that these latter samples had mutations at R172 of *IDH2*, a mutation previously identified in patients with central nervous system tumors. Moreover, recombinant mutant IDH2, but not wild-type IDH2, resulted in 2-HG accumulation. These data demonstrate that *IDH2* mutations occur in AML patients, and are associated with elevations in 2-HG similar to those seen in *IDH1* mutant AML and glioma patient samples.

In a concurrent study, Ward et al. (2010) screened a large cohort of AML patients for 2-HG production, and identified *IDH2* mutations in patients expressing wild-type *IDH1* but showing elevated 2-HG levels. In addition, Ward et al. (2010) identified a novel mutation in *IDH2* at residue R140 (R140Q), which was not seen previously in glioblastoma or AML patients, and which results in accumulation of 2-HG. The *IDH2* R140Q mutation is more common in AML than *IDH1* mutations or *IDH2* R172 mutations, suggesting that different mutations in *IDH1* and *IDH2* occur at different frequencies in different tumor types. They also performed structural modeling to show that these three residues in IDH1 and IDH2 that are mutated in AML patients are likely critical in stabilizing the interaction between IDH1 and IDH2

and their physiological substrate, isocitrate. Lastly, they performed cell culture studies to demonstrate that expression of IDH1 or IDH2 mutants results in increased α-ketoglutarate [α-KG]–dependent production of 2-HG.

Underlying this discovery of the production of 2-HG by mutant IDH1/2 enzymes in AML is the finding that mutant IDH1/2 enzymes also lead to consumption of NADPH (Dang et al., 2009). Normally, when IDH1/2 enzymes convert isocitrate to α-KG, this leads to the concurrent reduction of NAD(P)+ to NADPH. In fact, IDH1 is normally responsible for generating a significant proportion of cellular NADPH (Kil et al., 2006, 2007). NADPH is critical for several cellular metabolic processes, including synthesis of glutathione, which protects cells from oxidative stress. Thus, it is plausible that mutations that result in impairment of normal IDH1/2 function may deplete cellular NADPH. At the same time that *IDH1/2* mutations may reduce levels of NADPH, the metabolite created by the neomorphic function of mutant IDH1/2, 2-HG, is thought to increase levels of intracellular ROS (Kölker et al., 2002; Latini et al., 2003). As *IDH1/2* mutations result in depletion of NADPH and increased ROS, levels of α-KG are also decreased by IDH1/2 mutations. Because α-KG normally serves to activate proline hydroxylases that inactivate HIF-1α, depletion of α-KG results in increased levels of HIF-1α and its associated targets (MacKenzie et al., 2007). Thus, the abrogation of the normal enzymatic activity of IDH1/2, together with the acquisition of the ability to produce 2-HG, increases ROS abundance and HIF-1α stability.

The evidence linking accumulation of 2-HG to elevations in ROS comes from studies of primary neuronal cultures exposed to 2-HG in vitro and brain tissue from rats treated with 2-HG in vivo. In both settings, 2-HG treatment elicited ROS as measured by oxidant-sensitive dyes, lipid peroxidation, and/or levels of enzymatic oxidative stress defenses (Kölker et al., 2002; Latini et al., 2003). Further work to evaluate effects of 2-HG on ROS levels in tissues other than neuronal cells has yet to be performed. Moreover, studies to compare ROS levels in primary AML blasts with and without *IDH1/2* mutations will be necessary to establish a link between IDH1/2 mutations and elevation in ROS in LSCs and in the bulk leukemic clone.

All genomic data published thus far has indicated that mutations in *IDH1/2* occur as heterozygous missense mutations (Dang et al., 2009; Mardis et al., 2009; Yan et al., 2009; Zhao et al., 2009; Gross et al., 2010; Ward et al., 2010). The fact that homozygous missense mutations have not been identified is consistent with the finding that *IDH1/2* missense mutations result in a gain of function. Moreover, NAPDH produced by wild-type IDH1 enzymatic activity is a critical substrate for the neomorphic enzymatic activity of the mutant IDH1 (Ward et al., 2010). In addition, siRNA-mediated depletion of wild-type IDH1 or IDH2 is lethal to cells in culture, suggesting that the wild-type allele must be retained in *IDH1/2* mutant cancer cells.

The overall effect of concomitant increased ROS and HIF-1α activity in HSCs could affect quiescence and differentiation in a manner that promotes hematopoietic transformation (Fig. 1). The effect of metabolic derangements induced by *IDH1/2* mutations in the HSC might also be influenced by the presence of other oncogenic mutations seen in AML. Of note, mutational analysis of an AML cohort for *IDH1* R132, *IDH2* R172, and *IDH2* R140 mutations found *IDH1* and *IDH2* mutations in 8 and 15% of AML patients, respectively, demonstrating these are relatively common mutations in AML. Moreover, Gross et al. (2010) and Ward et al. (2010) suggest that *IDH1/2* mutant AML frequently occur in normal karyotype AML without other known oncogenic mutations. These results are corroborated in at least one other larger clinical study of *IDH1* mutations in AML samples (Chou et al., 2010). Clearly, larger studies with more clinical samples including both *IDH1* and *IDH2* mutational analysis will be required to delineate the mutational spectrum, prognostic effects, and associated clinical features of *IDH1/2* mutations in AML. However, the genetic data from these studies suggest a potential novel pathway to leukemic transformation for *IDH1/2* mutant hematopoietic progenitors. Notably, recent studies suggest that *IDH1/2* mutations can be found in AML transformed from myeloproliferative neoplasms, suggesting that these mutations can promote transformation of a preleukemic clone (Green and Beer, 2010; Abdel-Wahab et al., 2010).

In addition to genomic correlative studies of *IDH1/2* mutations in hematologic malignancy samples, experimental studies will elucidate the specific role of these mutations in leukemic transformation. Specifically, although the novel biochemical effects of these mutant alleles have been extensively described in recent studies, the mechanisms by which these mutations confer a selective advantage to the malignant clone are not yet understood. Examination of the effects of *IDH1/2* mutant alleles on hematopoietic ROS and HIF1-α levels, and on self-renewal, differentiation, and proliferation, will be critical. Likewise, study of the effects of expression of *IDH1/2* mutant alleles in the hematopoietic compartment will likely elucidate the mechanisms by which these alleles contribute to transformation. Nevertheless, taken together, these three new studies highlight the importance of metabolic pathways to the pathogenesis of AML, and suggest the possibility that elevated ROS levels in AML cells might represent an "Achilles heel" that can be used to specifically target AML cells with novel therapies.

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