Isocitrate dehydrogenase mutations in leukemia

Anna Sophia McKenney1,2 and Ross L. Levine1,3

1Human Oncology and Pathogenesis Program, 2Weill Cornell/Rockefeller/Sloan-Kettering Tri-Institutional MD-PhD Program, and 3Leukemia Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York, USA.

Recent genome-wide discovery studies have identified a spectrum of mutations in different malignancies and have led to the elucidation of novel pathways that contribute to oncogenic transformation. The discovery of mutations in the genes encoding isocitrate dehydrogenase (IDH) has uncovered a critical role for altered metabolism in onco-
genesis, and the neomorphic, oncogenic function of IDH mutations affects several epigenetic and gene regulatory
pathways. Here we discuss the relevance of IDH mutations to leukemia pathogenesis, therapy, and outcome and how
mutations in IDH1 and IDH2 affect the leukemia epigenome, hematopoietic differentiation, and clinical outcome.

Introduction

Mutations in isocitrate dehydrogenase (IDH) have been identified in a spectrum of human malignancies. Mutations in IDH1 were first identified in an exome resequencing analysis of patients with colorectal cancer (1). Shortly thereafter, recurrent IDH1 and IDH2 mutations were found in patients with glioma, most commonly in patients who present with lower-grade gliomas (2). IDH1 muta-
tions were subsequently discovered in patients with acute myeloid leukemia (AML) through whole genome sequencing (3), which was followed by the identification of somatic IDH2 mutations in patients with AML (4–6). Further studies revealed that IDH mutations induce a neomorphic function to produce the oncometabo-
lite 2-hydroxylutarate (2HG) (7, 8), which can inhibit many cellular processes (9, 10). In particular, the ability of 2HG to alter the epigenetic landscape makes IDH a prototypical target for prognostic
studies and drug targeting in leukemias.

Neomorphic function leads to oncometabolite production

IDH proteins catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (αKG, also known as 2-oxoglutarate). IDH3 pri-
marily functions as the allosterically regulated, rate-limiting enzymatic step in the TCA cycle, while the other two isoforms, which are mutated in cancer, utilize this catalytic process in additional
contexts including metabolism and glucose sensing (IDH1) and regulation of oxidative respiration (IDH2) (11, 12). Loss-of-func-
tion mutations in other TCA cycle components have previously been identified in other types of cancer, specifically in mutations in fumarate hydratase (FH) and succinate dehydrogenase (SDH).
As such, many hypothesized that IDH1/2 mutations would result in loss of metabolic activity, and indeed, enzymatic studies con-
firmed that the mutant protein’s ability to perform its native func-
tion is markedly attenuated, as measured by reduced production of αKG or NADPH (13, 14).

However, the genetic data relating to these mutations were more consistent with gain-of-function mutation: all of the observed alterations are somatic, heterozygous mutations that occur at highly conserved positions, which appear to be functionally equiv-
alent between different isoforms. This discrepancy was resolved when metabolic profiling showed that the IDH1 mutant protein catalyzes a neomorphic reaction that converts αKG to 2HG. 2HG can be detected at high levels in gliomas harboring these muta-
tions (4), and the accumulation of 2HG was further found to be common to oncogenic IDH mutations (8). This finding indicated
that 2HG may serve as a potential functional biomarker of IDH mutation, and later, metabolomics analysis of 2HG content in
patient samples led to the identification of IDH2 mutations in leukemias (6). IDH mutant proteins have been proposed to form a heterodimer with the remaining wild-type IDH isoform (7, 8, 14), which is consistent with genetic data showing retention of the wild-type allele in IDH-mutant cancers.

2HG and tet family enzymes in leukemogenesis

The discovery of the neomorphic function of IDH opened the doors for true investigation into the implications of these muta-
tions and the resultant intracellular accumulation of 2HG. 2HG is thought to competitively inhibit the activity of a broad spectrum of αKG-dependent enzymes with known and postulated roles in oncogenic transformation. Some targets, such as the pro-
lyl 4-hydroxylases, have unclear implications in leukemia patho-
genesis. However, the recent demonstration that alterations in epigenetic factors occur in the majority of acute leukemias led
to investigations of the effects of 2HG on the jumonji C domain
histone-modifying enzymes and the newly characterized tet methyl-
cytosine dioxygenase (TET) family of methylcytosine hydroxy-
lases. Importantly, expression of IDH or exposure to chemically
modified, cell-permeable 2HG affects hematopoietic differen-
tiation, likely due to changes in epigenetic regulation that induce
reversible alterations in differentiation states (15).

TET1 was initially discovered as a binding partner of mixed-
lineage leukemia (MLL) in patients with MLL-translocated AML (16, 17). However, the function of the TET gene family and its role in leukemogenesis remained unknown until TET1 was shown to catalyze αKG-dependent addition of a hydroxyl group to methyl-
ated cytosines (18), which precedes DNA demethylation and results in altered epigenetic control (10, 18–24). TET enzymes have further been shown to catalyze conversion of 5-methylcytosine (5mC) to
5-formylcytosine (5fC) or 5-carboxycytosine (5cC) (25, 26). These data suggest that loss of TET2 enzymatic function can lead to aberrant
cytosine methylation and epigenetic silencing in malignant
settings. TET2 mutations were initially found in array-comparative
genomic hybridization and genome-wide SNP arrays, which identi-
fied microdeletions containing this gene in a patient with myelo-
proliferative neoplasm (MPN) and myelodysplastic syndrome
(MDS) (27). This discovery was followed by the identification of somatic missense, nonsense, and frameshift TET2 mutations in
patients with MDS, MPN, AML, and other myeloid malignancies

Conflict of interest: Ross L. Levine reports receiving research funding from Agios Pharmaceuticals.

Citation for this article: J Clin Invest. 2013;123(9):3672–3677. doi:10.1172/JCI67266.
(27–30). Most TET2 alleles result in nonsense/frameshift mutations, which result in loss of TET2 catalytic function (31), consistent with a tumor suppressor function in myeloid malignancies.

When 2HG was hypothesized to affect specific enzymatic processes in oncogenesis, AML patients were observed to be harboring IDH1/2 and TET mutations in a mutually exclusive manner (9). Of note, exploration into the functional relationship between these mutant IDH proteins and the function of TET2 ultimately suggested a role for 2HG in inhibiting TET enzymatic function. IDH- or TET2-mutant patient samples are characterized by increased global hypermethylation of DNA and transcriptional silencing of genes with hypermethylated promoters. Expression of these IDH-mutant alleles in experimental models was further observed to result in increased methylation, reduced hydroxymethylation, and impaired TET2 function (9). Finally, in biochemical assays, 2HG was shown to directly inhibit TET2 as well as other αKG-dependent enzymes (10). These data demonstrate that a key feature of IDH1/2 mutations in hematopoietic cells is to impair TET2 function and disrupt DNA methylation (Figure 1).

**Models of leukemia employing IDH/2HG and TET2**

Conditional loss of Tet2 expression in mice results in a chronic myelomonocytic leukemia (CML) phenotype and in increased hematopoietic self-renewal in vivo (32). Of note, in vitro systems have shown that TET2 silencing and expression of IDH1/2 mutant alleles leads to impaired hematopoietic differentiation and expansion of stem/progenitor cells (9). More recently, IDH1 (R132H) conditional knockin mice with hematopoietic-specific recombination were analyzed and found to have myeloid expansion, although they did not develop overt AML. This suggests that IDH mutations by themselves cannot promote overt transformation, and that additional genetic, epigenetic, and/or microenvironmental factors are needed to cooperate with mutant IDH alleles to promote hematologic malignancies. The hematopoietic defects included increased numbers of hematopoietic stem cells and myeloid progenitor cells, and a DNA methylation signature that was similar to observed patterns in primary AML patients with IDH1 mutations (33). While many models of IDH-mutant leukemia have shown potential, future models that incorporate the complexity seen in human patients are needed, as discussed below. More recently, the effects of IDH1/2 mutations on hematopoietic cell lines were replicated using exogenously applied 2HG, which was rendered permeable to the cell membrane by esterification. The Kaelin group used this system to dissect the role of 2HG in the αKG-dependent pathways that may be affected in IDH mutation, and to show that the effects are reversible (34). Tools such as these will help advance our understanding of the biology of IDH mutations and, by extension, the potential therapies that may affect mutant IDH and the downstream pathways. Indeed, given the recent description of mutant-selective IDH1/2 inhibitors (34–37), the development of genetically accurate models of IDH mutant-mediated leukemogenesis will be critical to evaluate the effects of targeted therapies in mice with AML and subsequently in the clinical context.

### Table 1

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Prevalence hotspot mutations</th>
<th>N</th>
<th>Notes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>De novo AML</td>
<td>IDH1</td>
<td>16%</td>
<td>80 CN-AML</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>IDH2</td>
<td>14%</td>
<td>358 CN-AML</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6%</td>
<td>199 72 patients included with abnormal karyotype</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.5%</td>
<td>493</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.9%</td>
<td>68 Compared with other hematologic diseases</td>
<td>38</td>
</tr>
<tr>
<td>Pediatric AML</td>
<td>NT</td>
<td>0%</td>
<td>53</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td>1.7%</td>
<td>180</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td>2.2%</td>
<td>460 Most common in FAB-M1/M2 AML</td>
<td>48</td>
</tr>
<tr>
<td>MPN</td>
<td>2.5%</td>
<td>200</td>
<td></td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>1.2%</td>
<td>1,473</td>
<td></td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>1.6%</td>
<td>301</td>
<td>IDH mutation was correlated with reduced leukemia-free survival</td>
<td>45</td>
</tr>
<tr>
<td>MDS/MPN</td>
<td>0%</td>
<td>19</td>
<td></td>
<td>38</td>
</tr>
<tr>
<td>Secondary AML transformed from MPN</td>
<td>13.2%</td>
<td>53</td>
<td></td>
<td>43, 44</td>
</tr>
<tr>
<td>CML</td>
<td>3.7%</td>
<td>54</td>
<td>All were found in blast-phase CML</td>
<td>52</td>
</tr>
<tr>
<td>CMML</td>
<td>2.9%</td>
<td>70</td>
<td></td>
<td>40</td>
</tr>
<tr>
<td>Pediatric ALL</td>
<td>6.5%</td>
<td>31</td>
<td></td>
<td>53</td>
</tr>
</tbody>
</table>

NT, not tested.

Prevalence of IDH mutations in leukemia patients

IDH mutations are most common in patients with cytogenetically normal AML (CN-AML), and the prevalence of IDH1 and IDH2 missense mutations among patients with AML is between 5% and 20% (Table 1). In comparison with other cancers in which IDH1 mutations are more common than IDH2 mutations, IDH2 mutations are relatively more common in AML, such that the frequency of IDH1 and IDH2 are comparable in AML patients (38). IDH1 and IDH2 mutations are mutually exclusive from one another in AML.

Different subtypes of AML have been postulated to have different biases toward IDH mutation. For instance, some studies suggest that IDH1 mutations are more common in patients with abnormal karyotype, while IDH2 mutations are primarily found in patients with CN-AML (39). In addition, 3% of patients with CML were observed to have IDH1 mutations (40).

Patients can present with de novo AML, or alternatively can progress to AML from chronic-phase MPN and MDS. IDH mutations are associated with trisomy 8 in MDS and AML patients, but
IDH mutations are not as broadly characterized in other hematopoietic disorders. In chronic myeloid leukemia (CML), mutant IDH1 has been suggested to play a role in progression to blast phase of advanced disease (52). Examination of acute lymphoblastic leukemia (ALL) has also shown limited prevalence of IDH mutation, including 3%–5% of adult ALL and 0% of childhood ALL (53, 54).

**Prognosis for patients with IDH mutations**

The prognostic significance of IDH mutations is of great interest in leukemia, particularly given that IDH mutations are associated with relatively favorable outcome in gliomas. In AML, patients with the R132H IDH1 mutation have lower overall survival (55, 56). Patients with R172K IDH2 mutation have lower rates of complete remission (5), and patients with this mutation have a worse prognosis than those with R140Q IDH2 mutations (57). However, the relevance of IDH mutations to outcome in AML is more complex than it first appears. In one study of newly diagnosed patients, IDH mutations were associated with older age and higher platelet levels, but were not independent predictors of survival (58). IDH2 mutations have no influence on treatment outcome in CN-AML (59), and IDH1 mutations appear to remain stable over the course of disease, are not acquired at relapse, and do not have any independent impact on survival (60). In childhood AML, IDH mutation was actually associated with increased overall survival, but this covariate was not found to be an independent predictor of survival (48). Of note, two recent studies have suggested that R140Q IDH2 mutations are associated with favorable outcome in younger adults treated with dose-intensive therapy (57, 61), suggesting that the prognostic relevance of IDH mutations may depend on the specific allele, patient age, or treatment regimen. By contrast, IDH mutations, particularly R172K IDH2 mutations, are associated with adverse outcome in older adults with AML, suggesting an age-dependent relevance of IDH mutations to outcome in AML (5).

Although the independent prognostic value of IDH mutations themselves varies in different studies, IDH has been defined as part of prognostic subsets in combination with other mutations in AML. IDH mutation has often been observed to cooccur with mutations in the nucleophosmin (NPM1) protein in adults...
Since the neomorphic function of IDH was identified, 2HG has been considered as a potential biomarker of IDH-associated oncogenesis. Several studies have employed liquid-chromatography mass spectrometry to explore this. A prospective study of patients with newly diagnosed AML serially examined 2HG levels in serum and urine, with parallel evaluation of IDH1/2 mutant allele burden in bone marrow. Both of these parameters correlated with treatment response, suggesting that 2HG might serve as an appropriate proxy for IDH mutation and as a marker of residual disease (69). Recently, DiNardo and colleagues measured serum 2HG levels in patients from the E1900 AML patient cohort and found that 2HG correlated with the presence of IDH1/2 mutations, regardless of the specific mutant allele (73). Consistent with the mutational data, high 2HG levels with cooccurring NPM1 mutations or without cooccurring DNMT3a mutations were associated with improved survival (73). Most importantly, residual 2HG detected in samples taken when patients were in clinical remission were associated with impaired survival, suggesting that 2HG may serve as a sensitive measure of minimal residual disease in IDH-mutant AML.

As 2HG emerges as a biomarker, alternative assays have been developed for more convenient measurement. An enzymatic assay for 2HG has been developed that assesses levels of NADH, which is produced stoichiometrically as 2HG is metabolized by recombiant 2HG dehydrogenase (74). Alternatively, given the well-explored relationship between 2HG production and TET2 function, hydroxymethylcytosine (5hmC) levels have been found to correlate with IDH mutational status and response to treatment in leukemia patients, indicating that hydroxymethylation of genomic DNA may serve as a biomarker of IDH mutation activity in AML (75).

**IDH inhibitors**

The development and preclinical validation of IDH inhibitors has emerged as an important goal in order to test the viability of mutant IDH1/2 as a therapeutic target and as a tool to help dissect the IDH/2HG pathway in different malignant contexts. One compound under investigation is compound 35, which is active against R132H IDH1 mutants, and preclinical tests have indicated that its application reduces 2HG production in cell lines and mouse xenograft models (35). A similar selective compound was more recently used in combination with an exogenous 2HG system to show that the cellular phenotype induced by IDH mutation is reversed by this drug, whereas the cellular phenotype induced by exogenous 2HG production is not (34). Recently, compounds targeting mutant IDH2 have been explored in leukemia cell lines (36) and glioma cell models (37). In both cases, IDH inhibitors were found to reduce 2HG production and inhibit the growth of leukemia or glioma cells in a mutant-specific manner. In addition, IDH inhibition led to global changes in DNA methylation/histone state and to induction of hematopoietic/neural differentiation, suggesting that these agents might induce differentiation in IDH-mutant cells through alterations in the epigenetic state. However, extensive in vivo studies in IDH-mutant transformation models remain to be reported, and the role of IDH in malignant cells after oncogenic transformation requires additional, extensive investigation.

**IDH mutations in leukemia**

The discovery of mutation in metabolic genes has given long-awaited credence to the concept that altered metabolism is a hallmark of human cancers. However, the implications of mutations...
in genes such as IDH to oncogenesis are complex, and enormous potential lies in understanding the ramifications of this mutation, 2HG production, and downstream effectors such as TET2 in leukemia. Understanding this system will require the production of better models of IDH-mutant leukemias that incorporate the genetic complexity observed in human leukemia, particularly incorporation of many combinations of mutations. Clinically, the significance of IDH mutation must be examined in the context of other mechanistically important factors in larger cohorts with more comprehensive mutational and epigenetic analysis—and new technologies in screening, sequencing, and otherwise evaluating patient samples will help allow this to happen. As IDH inhibitors continue to be developed and to move closer to clinical testing, it will be come paramount to identify patient populations that stand to benefit the most from IDH-targeted therapy and to determine whether combination therapies should be investigated. Finally, while the role of IDH mutations in prognosis and therapeutic response requires further evaluation, there is clearly significant evidence that understanding its function in this disease will unlock significant mechanistic features of leukogenesis. Particularly given its differential prognostic impact in glioma, a deeper understanding of these mutations and their neomorphic, oncogenic function will improve our understanding of carcinogenesis.

Acknowledgments
A.S. McKenney was supported by NIH MSTP grant GM07739.

Address correspondence to: Ross L. Levine, Human Oncology and Pathogenesis Program, Leukemia Service, Department of Medicine, 1275 York Avenue, Box 20, New York, New York 10065, USA. Phone: 646.888.2796; Fax: 646.422.0890; E-mail: leviner@mskcc.org.

17. Lorsbach RB, Moore J, Mathew S, Raimondi SC, Mukatira ST, Downing JR. TET1, a member of a novel protein family, is fused to MLL in acute myeloid leukemia containing the t(10;11) (q22;q23). Leukemia. 2003;17(3):637–641.


