Kinase Networks Regulate Metabolism: I’D(H1) Never Have Guessed!

Sarah Horton and Brian J.P. Huntly

**Summary:** Mutations in isoforms of isocitrate dehydrogenase (IDH) enzymes are described in multiple cancers and both mutant and wild-type IDH are important for the generation and maintenance of tumors, but how their activity is regulated is poorly understood. An article in this issue of Cancer Discovery identifies a novel post-translational mechanism of IDH1 regulation involving phosphorylation of specific tyrosine residues by a network of kinases that alter the specificity of substrate and cofactor binding, dimer formation, and ultimately enzyme activity.

See related article by Chen et al. p. 756 (8).

Cancer cells have increased requirements for energy and biomass production, and these requirements are served through alterations of their metabolic circuitry (1). Central nodes within the circuitry of both normal and malignant cells are the enzymes isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2). These enzymes are located within the cytoplasm and mitochondria, respectively, and normally catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (αKG, also known as 2-oxoglutarate) in the Krebs cycle, a stepwise reaction that also produces CO2 and NADPH. αKG is an intermediate in both the Krebs and glutaminolysis cycles, an important nitrogen transporter for amino acid synthesis and an intermediate in both the Krebs and glutaminolysis cycles, an important nitrogen transporter for amino acid synthesis and fatty-acid chain elongation. Recently, cancer-associated mutations in both IDH1 and IDH2 have been described across a number of malignancies, including gliomas, glioblastomas, and acute myeloid leukemia (AML; refs. 3, 4). These mutations are located within the cytoplasm and mitochondria, respectively, and normally catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (αKG, also known as 2-oxoglutarate) in the Krebs cycle, a stepwise reaction that also produces CO2 and NADPH. αKG is an intermediate in both the Krebs and glutaminolysis cycles, an important nitrogen transporter for amino acid synthesis and an alteration of gene expression that contributes to the malignant phenotype (2, 5). Recently small-molecule inhibitors of IDH1 and IDH2 have been developed that have demonstrated considerable preclinical efficacy (6, 7). This promise has been vindicated in early-phase trials and, within the last two years, enosidenib (AG-221) and ivosidenib (AG-120) have been approved by the FDA for the treatment of IDH2- and IDH1-mutant AML, respectively. However, how mutant and wild-type (WT) IDH proteins are further regulated is poorly understood.

In a very interesting article in this issue, Dong Chen and colleagues, from the laboratories of Jing Chen and Ross Levine (8), describe and characterize a novel mechanism regulating IDH1 activity. This occurs at the post-translational level and involves phosphorylation of both the WT and mutant IDH1 proteins on a specific tyrosine by a communicating network of kinases (Fig. 1). Their initial observation built on previous findings of synergistic efficacy in preclinical models when AG-221 was combined with a FLT3 inhibitor (9). Interestingly, when the authors assessed similar synergy between the IDH1 inhibitor AG-120 and the FLT3 inhibitor quizartinib in primary AML blasts across a range of patient genotypes (WT or mutant IDH1 and either mutant or WT FLT3), to their surprise they noted that both AG-120 and quizartinib reduced the biochemical activity of IDH1. The authors then linked this to tyrosine phosphorylation of IDH1 using *in vitro* kinase assays, where they could demonstrate a phosphorylation-dependent increase in enzymatic activity upon incubation with a number of recombinant kinases. Performing the same assays with recombinant IDH1 systematically mutagenized to alter tyrosine residues (Y → F), they identified critical residues to be Y42 and Y391 and could further link a group of tyrosine kinases (TK; which they called Group I kinases) including EGFR, JAK2, ABL1, PDGFR, and FGFR1 to phosphorylation of Y42 and a separate group of kinases (Group II kinases), including Src and FLT3, to Y391 phosphorylation. Moreover, biochemical and later chemical inhibition experiments could demonstrate a reciprocal hierarchical/network relationship between the Group I and Group II kinases, with evidence of Group I activating Group II kinases (i.e., FGFR1/ABL1 activating Src kinases) or vice versa (i.e., FLT3 activating JAK2).
To understand the mechanisms of altered enzyme function upon Y42 and Y391 phosphorylation, they next performed sophisticated in vitro biochemical analyses. These determined that dimerization of IDH1 increased the enzymatic activity of WT and mutant-containing dimers, and also that phosphorylation of Y42 led to an increase in both dimer formation and stability. Y42 phosphorylated IDH1 (WT and mutant) also bound more avidly to labeled isocitrate and αKG, substrates for WT IDH1 and mutant IDH1, respectively. Conversely, Y391 phosphorylated IDH1 did not alter dimerization or substrate binding, but instead demonstrated increased binding to NADP+ and NADPH, the cofactors for the oxidative carboxylation reaction of WT IDH1 and the further reduction performed by mutant IDH1. Functionally, abrogating tyrosine phosphorylation at these critical tyrosine residues led to growth retardation of both mutant IDH1 AML and WT IDH1 lung cancer cells. This phenotype was linked to a decrease in IDH enzymatic activity, and also to reduced 2-HG levels and histone hypermethylation in the context of IDH1-mutant AML. Finally, the authors confirmed their findings in primary AML cells from patients, irrespective of their IDH1 and FLT3 mutational status, demonstrating that tyrosine phosphatase or FLT3 inhibitor treatment of either mutant or WT IDH1 cells reduced Y42 and Y391 levels, IDH1 dimerization, and enzymatic activity in all cells and 2-HG levels in mutant cells. They also corroborated their findings across tissue arrays from a wide range of human tumors, demonstrating an increased Y42 phosphorylation of IDH1 protein in comparison with normal control tissue.

On a basic level, these studies demonstrate a novel link between intracellular signaling and central metabolism. As this communication requires neither mutant IDH1 nor mutant kinases, it is likely that it represents a normal physiologic response, coupling increased energy and biomass provision to proliferation, and it is not surprising that this process is hijacked during malignant transformation. It will be of interest to determine whether other critical metabolic master regulators are similarly modulated in normal physiology, and whether this process is co-opted in malignant transformation. Of note, the mechanisms described seem highly specific for IDH1; they cannot occur at the corresponding residues in IDH2, where tyrosine residues are replaced by phenylalanines.
This, in turn, asks the question of whether similar post-translational phosphorylation events occur as a regulatory mechanism for IDH2 and, if so, at which critical residues and by which kinases? The same authors, along with others, have previously shown that TKs do function in the mitochondria to alter the function of master regulators of metabolism such as pyruvate dehydrogenase (10). However, although TKs such as ABL1 and some Src kinases have been demonstrated to be active in the mitochondria, general TK activity is less in this compartment. Therefore, the identity of any regulatory kinases is likely to differ between cytosol and mitochondria and will need to be investigated.

These data also suggest that further studies are warranted to determine the functional consequences of phosphorylation of other tyrosine residues within IDH1. For example, the authors cite the proposed critical role of tyrosine 139 (Y139) for full catalytic activity of IDH1. However, it is not currently known whether this role involves direct phosphorylation of Y139. Other important questions relate to the mechanisms downstream of Y42 and Y391 phosphorylation and to their exact stoichiometry. These include the following: What is the specific structural basis of increased dimer formation, dimer stability, and increased substrate binding for Y42 phosphorylation? Similarly, what is the structural basis that explains the increase in cofactor binding upon Y391 phosphorylation? Regarding stoichiometry, do both members of the dimer, or only one, need to be phosphorylated on Y42 and Y391, and what is the role for compound (Y42/391) phosphorylation? To answer these questions, further detailed structural and mechanistic studies will be required. The findings of Chen and colleagues also have therapeutic implications. They suggest that the efficacy of TK inhibitors in tumors may relate, at least in part, to their modulation of IDH1 metabolic function. Furthermore, they provide a further rationale for combining IDH1 inhibition with TK inhibitors in patients with IDH1-mutant tumors, where both metabolic and epigenetic abnormalities are targeted. This rationale is most compelling in AML, where tyrosine signaling from both WT and oncogenic mutant TKs has been shown to contribute to the malignant phenotype.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

Published online June 3, 2019.

**REFERENCES**


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