kinases such as CDK4, monoclonal antibodies targeting receptors like IGF-R1 or EGFR, and peptide-mimetic MDM2 protein-interaction inhibitors that can rescue wt-p53 functions (Figure 1). We can now include the clinically relevant p53 protein interaction from Twist1 as a key target to evaluate for its therapeutic potential. There is enormous promise in drugging such protein-protein interactions, as this forms an untapped and large landscape for drug development (Crunkhorn, 2011).

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IDH1 Mutations Disrupt Blood, Brain, and Barriers

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The first two murine models of IDH1(R132H) mutation provide mechanistic insights into transformation. In hematopoietic cells, inhibition of TET2 and histone demethylases leads to epigenetic alterations and accumulation of hematopoietic precursors. In the central nervous system, inhibition of collagen and prolyl hydroxylases lead to altered microenvironment and defective angiogenesis.

Large-scale sequencing efforts have identified novel genes that are somatically mutated in cancer. Two of the more unexpected genes that have been implicated as recurrent mutational targets are *IDH1* and *IDH2*, which encode isocitrate dehydrogenase-1 and isocitrate dehydrogenase-2, respectively. These enzymes catalyze the conversion of isocitrate to α-ketoglutarate (αKG) in an $NADP^+$ dependent manner. Mutations in *IDH1* were first identified in colorectal cancer, and later, mutations in *IDH1*/*IDH2* were identified in brain tumors, with >70% incidence in secondary gliomas (Yan et al., 2009). Through whole-genome sequencing of a case of acute myeloid leukemia (AML), an *IDH1* mutation was identified, and *IDH1*/*IDH2* mutations were subsequently found in 12%–18% of AML cases (Mardis et al., 2009). Mutations have also been identified in thyroid cancers, chondrosarcomas, and cholangiocarcinomas.

At first it was puzzling how basic metabolic enzymes could be linked to cancer. The mutant IDH1/IDH2 enzymes have decreased enzymatic activity known at that time. However, the observations that the mutations always presented as heterozygous and at highly conserved arginine residues are more consistent with these being gain-of-function. This led to the critical finding that these mutants acquired neomorphic activity converting αKG to 2-hydroxyglutarate (2HG) (Dang et al., 2009; Ward et al., 2010). Tumor samples harboring these mutations had 2HG at levels up to ~100-fold greater than controls. Besides being an intermediate in the Krebs cycle, αKG is involved in other biochemical processes, including synthesis of glutamate, transamination of amino acids, generation of NADPH, and acting as a cofactor for dioxygenase enzymes. The structural similarity between 2HG and αKG suggested that other enzymatic processes may be competitively inhibited by elevated 2HG levels (Xu et al., 2011) (Figure 1).

Through analysis of global DNA methylation profiles in glioblastomas (GBMs), a distinct profile termed CpG island methylator phenotype with elevated genomic methylation was found to be closely associated with *IDH1* mutations (Noushmehr et al., 2010). Subsequently, it was discovered that *IDH1*/*IDH2* mutations were mutually exclusive with *TET2* mutations, a gene encoding an αKG-dependent enzyme involved in DNA demethylation, suggesting that these proteins are involved in the same pathway. Biologic significance was demonstrated through

bone marrow transplant assays in which expression of IDH1/IDH2 mutant proteins or functional loss of TET2 resulted in similar changes that promote leukemogenesis (Figueroa et al., 2010). More recent work has also identified histone demethylases as potential αKG-dependent targets (Lu et al., 2012).

Two recent papers published by Sasaki et al. report the phenotype of mice expressing the IDH1(R132H) mutant from the endogenous *Idh1* locus. The first paper reports the phenotype of mice expressing IDH1(R132H) targeted to the hematopoietic system (Sasaki et al., 2012a). These mice develop hematologic abnormalities, including anemia, splenomegaly, and extramedullary hematopoiesis. These mice are characterized by hematopoietic stem/progenitor expansion, including increased numbers of LSK ($Lin^{-}Sca1^{+}cKit^{+}$) cells and lineage restricted progenitors ($CD150^{-}CD48^{+}$). Using enhanced representation bisulfite sequencing, the authors found that LSK cells from these mutant mice had significantly more methylated CpG sites compared to control LSK cells, most notably at promoter and intragenic regions. This increased global methylation is hypothesized to be secondary to inhibition of TET2. Likewise, they find increased histone methylation, particularly for H3K4me3 and H3K79me2, chromatin marks that are associated with active or poised transcription. Inhibition of Jumonji-C domain-containing histone demethylases by 2HG likely underlies this observation. These findings agree with results in *IDH1*/*IDH2* mutant AMLs that have a hypermethylation profile with a significant overlap in commonly hypermethylated genes. They also extend our mechanistic understanding of leukemogeneisis. In the subset of AMLs caused by translocations of the mixed lineage leukemia (*MLL*) gene, it is believed increased H3K79me2 activity through recruitment of DOT1L contributes to dysregulated gene expression, and it is possible that inhibition of DOT1L might reverse the phenotypic effects of mutant *IDH* alleles on hematopoietic function.

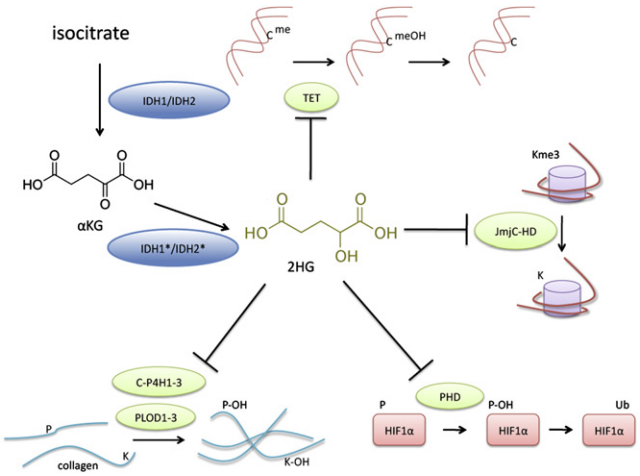

Figure 1. IDH1 Mutant Disrupts Multiple Cellular Processes
IDH1(R132H) produces 2HG, which inhibits αKG dependent dioxygenases. Inhibition of TET enzymes blocks 5-hydroxy methylcytosine modification, which leads to cytosine demethylation. Inhibition of JmjC-HDs leads to altered histone methylation on multiple histone H3 lysine residues. Inhibition of C-P4H1-3 and PLOD1-3 prevents proper prolyl and lysyl hydroxylation of collagen, which are required for their proper maturation. Inhibition of PHDs prevents HIF1α prolyl hydroxylation and subsequent ubiquitination by the von Hippel Lindau protein and degradation by the proteasome. A combination of these effects leads to epigenetic changes in the bone marrow, resulting in altered differentiation of hematopoietic progenitors and dysfunctional angiogensis in the brain, resulting in CNS hemorrhage. IDH1/IDH2 isocitrate dehydrogenase-1 and isocitrate dehydrogenase-2, IDH1*/IDH2* mutant IDH1 or IDH2; αKG, α-ketoglutarate; 2HG, 2-hydroxygluatare; TET, Tet methylcytosine dioxygenase; JmjC-HD, Jumonji-C domain-containing histone demethylases; C-P4H1-3, collagen prolyl 4-hydroxylase 1-3; PLOD1-3, procollagen-lysine, 2-oxoglutarate 5-dioxygenases 1-3; PHD, prolyl hydroxylase domain-containing proteins; HIF1α, hypoxia-inducible factor 1α; C, cytosine; P, proline; K, lysine; Ub, ubiquitin; me, methyl; OH, hydroxy.

In the second paper, Sasaki et al. (2012b) describe the phenotype of mice expressing IDH1(R132H) in the CNS using two cre strains (*Nestin* and *GFAP*). These mice develop perinatal lethality due to intracerebral hemorrhage. They noted variable penetrance in the *GFAP-cre* line; phenotypic severity correlated with levels of 2HG expression. This abnormal vascular development appears to be a consequence of 2HG-mediated inhibition of the dioxygenases that regulate collagen maturation and hypoxia-inducible transcription factors (HIF)1α and HIF2α degradation. Type IV collagen is a component of the basement membrane between astrocytes and endothelial cells. Its maturation depends on hydroxylation by procollagen-lysine, 2-oxoglutarate 5-dioxgenases 1-3 (PLOD1-3) and collagen prolyl-4-hydroxylases 1-3 (C-P4H1-3). In *Idh1* mutant mice, immature forms of collagen accumulate, and collagen fails to properly deposit along blood vessels. Accumulation of immature collagen also leads to a secondary endoplasmic reticulum (ER) stress response, which may be responsible for the increased apoptosis seen in the brain of mice expressing mutant *Idh1*. In addition, HIF1α/HIF2α are regulated by prolyl-hydroxylation, which leads to degradation by the ubiquitin-proteasome pathway through von Hippel Lindau (VHL) protein-mediated E3 ligase activity. The prolyl hydroxylase domain-containing proteins (PHDs) that regulate HIF1α/HIF2α stability belong to the dioxygenase family and are likewise another potential target of 2HG inhibition. In mutant mice, HIF1α accumulates, and the expression of HIF1α target genes, including *Vegf*, concomitantly increases. The combination of defective vessel integrity, increased ER stress, and elevated angiogenic signaling, likely leads to the intracerebral hemorrhage seen with brain-specific expression of IDH1(R132H).

Unlike the hematopoietic model, the CNS model of IDH1(R132H) expression does not suggest a putative mechanism for how glial cells expressing mutant IDH are transformed. Although abnormal angiogensis is a hallmark of GBM and brain tumor progression, *IDH1* mutations are an early event, with mutations detected more frequently in low-grade gliomas and secondary GBMs than in primary GBMs (Yan et al., 2009). Contrary to their expectation, the authors noted lower levels reactive oxygen species in mutant brain cells compared to controls. In contrast to the hematopoietic model, the authors did not note significant changes in histone methylation in the brain. Furthermore, although there was a decrease in 5-hydroxymethylcytosine (the product of TET2 enzymatic activity), the authors did not provide evidence of

changes in global methylation. This is reflected in the lack of changes in the differentiation pattern of CNS stem cells and failure of these mice to show evidence of glioma formation even with age.

These mouse models serve as an important step in modeling oncogenic *IDH* alleles and will undoubtedly serve as the basis for studies combining oncogenic *IDH* alleles with other mutations known to co-occur with *IDH1*/*IDH2* mutations in AML, glioma, and other malignancies. These results also raise fundamental biochemical questions as to whether specific enzymes are differentially inhibited by 2HG in a tissue-specific manner, and which of the many enzymes inhibited by 2HG are essential for transformation. In addition, further work is needed to bring greater resolution as to how epigenetic repatterning at key genes contributes to oncogenic transformation. Taken together, these studies demonstrate that *IDH1*/*IDH2* mutations will have pleiotropic effects in different contexts that contribute to transformation in different tumor types. Moreover, the development of these models provides an avenue for preclinical testing of IDH1/IDH2 inhibitors, such that we learn whether this represents a potential therapy for the subset of patients with neomorphic *IDH* disease alleles.

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Tailor-Made Renal Cell Carcinoma Vaccines

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Cancer vaccines are beginning to show signs of clinical activity, but major uncertainties remain regarding antigen selection, strategy for immune stimulation, patient stratification, and monitoring of elicited response. A new study of peptide vaccines in advanced renal cell carcinoma patients provides important insights into these central issues.

Recent clinical successes have validated the long-standing idea that therapeutic manipulation of endogenous immunity may achieve meaningful anti-tumor effects (Mellman et al., 2011). Perhaps the most compelling evidence marshaled to date derive from studies of blocking monoclonal antibodies against key negative immune regulatory molecules, such as cytotoxic T lymphocyte-associated antigen-4 and programmed death-1, which achieve durable tumor regressions and/or disease control in several types of malignancies. These therapeutic approaches are limited in selectivity for amplifying anti-tumor immune responses, however, and thereby sometimes provoke serious inflammatory reactions in normal tissues.

Compared to targeting immune regulatory pathways, cancer vaccines offer a stronger potential for focusing immune reactions toward tumor-specific and tumor-associated antigens, but the clinical impact of these strategies thus far has been more modest, highlighting the need for further optimization (Mellman et al., 2011). Vaccines aim to load dendritic cells (DCs), the professional antigen presenting cells of the immune system, with relevant cancer antigens and stimulate DCs to mature and migrate to regional lymph nodes, where they may efficiently prime tumor antigen-specific T and B lymphocytes. The activated T cells, particularly cytotoxic $CD8^+$ lymphocytes but also $CD4^+$ effectors, may in turn traffic systemically to metastatic deposits and thereupon effectuate tumor