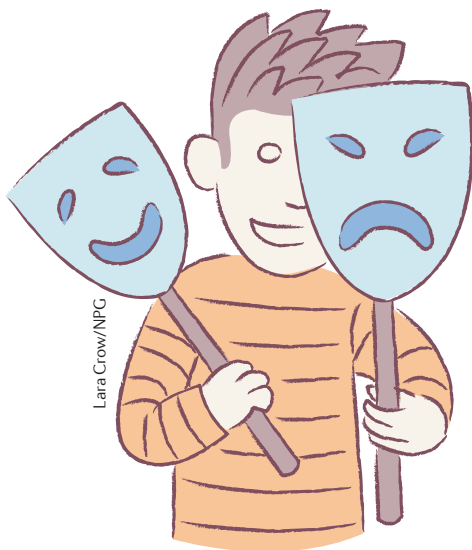➡ **METABOLISM**

Unmasking an oncometabolite

Lara Crow/NPG

Isocitrate dehydrogenase 1 (IDH1) and IDH2 are mutated in a subset of gliomas and leukaemias. Mutation of these enzymes endows them with a new function: the generation of 2-hydroxyglutarate (2HG) instead of α-ketoglutarate (α-KG). New data indicate that 2HG alters gene transcription through effects on DNA and histone methylation and hypoxia inducible factor 1α (HIF1α) and HIF2α.

Gliomas with IDH mutations have a CpG island methylator phenotype (CIMP), and to determine whether these facts are linked, Timothy Chan and colleagues compared the DNA methylation patterns in isogenic astrocytes expressing either wild-type IDH1 or mutant IDH1. Expression of mutant IDH1 caused changes in the DNA methylome: mainly hypermethylation with a few areas of hypomethylation. The changes in the methylome in mutant IDH1-expressing astrocytes mirrored the methylation patterns in CIMP-positive gliomas. Gene set enrichment analyses in lower grade gliomas (LGGs) identified a significant concordance in the affected genes between astrocytes expressing mutant IDH1 and gliomas with CIMP. In addition, a gene expression signature

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comprising 17 genes that are down-regulated in cells expressing mutant IDH1 was able to classify an independent set of LGGs into CIMP positive or negative, indicating that IDH mutations and the CIMP are closely associated. Chromatin immunoprecipitation experiments indicated that the genes that are hypermethylated in cells expressing mutant IDH1 are associated with alterations in H3K9 and H3K27 histone methylation, indicating that alterations in histone marks might contribute to DNA hypermethylation. These authors also found that astrocytes expressing mutant IDH1 develop a stem-cell-like phenotype in concert with DNA hypermethylation, indicating that mutant IDH1 might prevent differentiation.

A suppression of differentiation is reported in the paper by Craig Thompson and colleagues. Gene expression and gene ontology analyses indicated that astrocyte and glial cell differentiation genes were highly differentially expressed in cells with IDH mutations compared to those without. 2HG can function as a competitive inhibitor of α-KG-dependent Jumonji-C histone demethylases (JHDMs). Expression of either IDH1 or IDH2 mutants in 293T cells resulted in an increase in the levels of histone methylation, especially in the repressive histone marks H3K9me3 and H3K27me3. Moreover, the differentiation of mouse 3T3-L1 cells into adipocytes was inhibited by the expression of mutant IDH2 or the presence of a cell-permeable form of 2HG. These authors also noted that the levels of JMJD2C, an H3K9-specific JHDM, were increased in differentiating 3T3-L1 cells. An *in vitro* histone demethylation assay confirmed that JMJD2C removed H3K9me3 and H3K9me2 marks in the presence of α-KG, and this reaction was competitively inhibited by 2HG. Small interfering RNAs against JMJD2C also inhibited adipocyte differentiation, indicating that the removal of H3K9 methylation marks from histones is required for differentiation. Expression of mutant IDH1 in immortalized normal human astrocytes increased H3K9 methylation marks from passage 12, and the

expression of mutant IDH1 R132H in primary mouse neurosphere cultures prevented their differentiation.

In addition to the changes in DNA and histone methylation, William Kaelin and colleagues have found that unlike the *S*-enantiomer of 2HG, which is inhibitory, the *R*-enantiomer, which is the form produced by mutant IDH1 and IDH2, can promote the activity of the HIFα prolyl 4-hydroxy-lases (EGLN1, EGLN2 and, to a lesser extent, EGLN3). (*R*)-2HG did not activate other α-KG-dependent enzymes, including JMJD2D and the TET1 and TET2 methyl cytosine hydroxylases, all of which are inhibited by both (*S*)-2HG and (*R*)-2HG. Further investigations revealed that (*R*)-2HG functions as a co-substrate for EGLN1 in the prolyl hydroxyla-tion of HIF1α. Consistent with this, the stability of HIF1α and HIF2α was reduced in mid passage (9–15) human astrocytes expressing mutant IDH1, and a blunted HIF1α response to hypoxia was evident in cell lines derived from two oligodendrogliomas with IDH1 R132H mutations and in isogenic HCT116 colorectal cancer cell lines in which the R132H mutant was expressed from the endogenous *IDH1* locus. In addition, downregulation of HIF1α using short hairpin RNAs in immortalized human astrocytes increased their growth in soft agar, and a similar effect was seen when EGLN1 was overexpressed in these cells. Conversely, knockdown of EGLN1 in late passage cells expressing mutant IDH1 inhibited proliferation. Gene expression data from The Cancer Genome Atlas also showed that proneural tumours with IDH mutations had reduced expression levels of HIF-responsive genes. These authors conclude that the accumulation of (*R*)-2HG promotes transfor-mation through a mechanism that involves reduced HIF expression.

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ORIGINAL RESEARCH PAPERS Turcan, S. *et al.* IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. *Nature* 15 Feb 2012 (doi:10.1038/nature10866) | Lu, C. *et al.* IDH mutation impairs histone demethylation and results in a block to cell differentiation. *Nature* 15 Feb 2012 (doi:10.1038/nature10860) | Koivunen, P. *et al.* Transformation by the (*R*)-enantiomer of 2-hydroxyglutarate linked to EGLN activation. *Nature* 15 Feb 2012 (doi:10.1038/nature10898)