Photoactivation of Mutant Isocitrate Dehydrogenase 2 Reveals Rapid Cancer-Associated Metabolic and Epigenetic Changes

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Supporting Information

ABSTRACT: Isocitrate dehydrogenase is mutated at a key active site arginine residue (Arg172 in IDH2) in many cancers, leading to the synthesis of the oncometabolite ((R)-2-hydroxyglutarate (2HG)). To investigate the early events following acquisition of this mutation in mammalian cells we created a photoactivatable version of IDH2(R172K), in which K172 is replaced with a photocaged lysine (PCK), via genetic code expansion. Illumination of cells expressing this mutant protein led to a rapid increase in the levels of 2HG, with 2HG levels reaching those measured in patient tumor samples, within 8 h. 2HG accumulation is closely followed by a global decrease in 5-hydroxymethylcytosine (5-hmC) in DNA, demonstrating that perturbations in epigenetic DNA base modifications are an early consequence of mutant IDH2 in cells. Our results provide a paradigm for rapidly and synchronously uncloaking diverse oncogenic mutations in live cells to reveal the sequence of events through which they may ultimately cause transformation.

The citric acid cycle enzyme isocitrate dehydrogenase (IDH)1,2 catalyzes the NADP+-dependent oxidative decarboxylation of isocitrate to alpha-ketoglutarate (KGA) (Scheme 1a).

In a large proportion of cancers, including low grade gliomas, secondary glioblastoma multiforme, and acute myeloid leukemias, IDH is mutated at a key active site arginine residue. This arginine residue ordinarily forms hydrogen bond interactions with the alpha- and beta-carboxyl groups of isocitrate.3 Mutant cytosolic and peroxisomal IDH1 contains an Arg132His mutation (IDH1 R132H), while mutant mitochondrial IDH2, contains the analogous Arg172Lys mutation (IDH2 R172K). These mutations may hinder the enzyme’s interaction with isocitrate and increase its affinity for NADPH and KGA. The mutant forms of IDH catalyze an additional reaction: the NADPH-dependent reduction of KGA to the (R)-enantiomer of 2-hydroxyglutarate [(R)-2HG] (Scheme 1b).4−6 (R)-2HG has been proposed to be an oncometabolite, which together with the mutant enzyme is capable of driving cancer-associated cellular transformations.7,8 (R)-2HG is believed to drive transformation, in part, by competitively inhibiting KGA-dependent dioxygenase enzymes. Ten–eleven-translocation enzymes (TET) belong to this protein family and catalyze the conversion of the epigenetic DNA base 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) (Scheme 2).9−11 A decrease in global 5-hydroxymethylcytosine levels is characteristic of some cancers, including those with mutations in IDH.12,13 However, it has not been possible to temporally define the sequence of metabolic and epigenetic changes that immediately follow the acquisition of the potentially oncogenic mutations in IDH.

Strategies to photocage key residues within a target protein provide a powerful approach for creating inactive proteins that can be rapidly activated with a pulse of light. Amino acids have been targeted for photocaging by chemical ligation and introduced into cells by microinjection.14,15 More recently photocaged amino acids have been site-specifically and cotranslationally incorporated into proteins via genetic code expansion. Photocaged lysine, tyrosine, serine, and cysteine have been site-specifically incorporated in place of key residues in proteins.16−23

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Scheme 1. Chemical Conversions Catalyzed by Wild-Type (a) and Mutant (b) IDH

Scheme 2. TET Enzymes Catalyze the Conversion of 5-mC to 5-hmC and Further Oxidation Steps9,11

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The approaches developed have allowed photocontrol of nuclear localization sequences, and several enzymes, including kinases, proteases, inteins, and cas9, as well as photocontrol of the sites of post-translational modification. These approaches have revealed the kinetics of complex signaling pathways with high spatial and temporal resolution and provided approaches to spatially and temporally control tools for proteome and genome editing.

We have previously evolved a PCKRS/tRNA<sub>CUA</sub> pair, which directs the incorporation of photocaged lysine (PCK, Figure 1a) into target protein. This pair is derived from the pyrrolysyl-tRNA<sub>CUA</sub> pair, which is used for proteome and genome editing. Isolated clones exhibited clear PCK dependent read through of the amber stop codon in IDH2-R172TAG-GFP, as judged by flow cytometry and western blot (Figure 1c,d), consistent with the synthesis of IDH2(R172PCK)-GFP. IDH2-R172PCK-GFP was mitochondrial, as judged by colocalization of GFP fluorescence with MitoTracker staining (Figure 1e). The half-life of IDH2-R172PCK-GFP is approximately 48 h, providing an upper limit on the length of time after photoactivation for which measurements will result from a consistent level of mutant protein (Supplementary Figure S2).

To determine whether cells expressing IDH2(R172PCK)-GFP synthesize 2HG, we extracted metabolites from cells and quantified cellular 2HG levels by liquid chromatography, coupled to tandem mass spectrometry (LC-MS/MS), using an isotopically labeled internal standard. We found that 2HG levels were below the detectable threshold, demonstrating that IDH2(R172PCK)-GFP does not appreciably catalyze the NADPH-dependent reduction of KGA to 2HG.

Illumination (365 nm, 60 s, 9.5 mW/cm²) of cells expressing IDH2(R172PCK)-GFP led to the accumulation of 2.37 nmol of 2HG per 100 µg of protein, 8 h after illumination (Figure 2a).

This is equivalent to an approximate cellular 2HG concentration of 2.9 mM and is within the range of 2HG levels reported in both cells transfected with the mutant protein and patient tumor samples. This result demonstrates that photoactivation of IDH2(R172PCK)-GFP reveals IDH2(R172K)-GFP, which rapidly produces relevant levels of the oncometabolite (R)-2HG. The timecourse of (R)-2HG production following

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**Figure 1.** Site-specific incorporation of photocaged lysine at residue 172 of mitochondrial IDH2. (a) Photocaged lysine PCK, 1. (b) Caged lysine in the IDH2(R172PCK) active site is proposed to prevent the NADPH-dependent reduction of KGA to (R)-2HG. Illumination reveals the active mutant protein (the figure is a structural model created using Pymol, Phenix, and IDH2 structure PDB: 4JA8). (c) GFP fluorescence of HEK293 cells, stably expressing IDH2(R172TAG)-GFP and the PCKRS/tRNA<sub>CUA</sub> pair, in the presence (blue) and absence (red) of 0.2 mM PCK, 1. The data were collected by flow cytometry. (d) Immunoblot (IB) of cells from panel c with anti-GFP antibody confirms expression of IDH2-GFP fusion protein with a molecular weight of 78 kDa. Please see Supplementary Figure S1a for the full gel. (e) Mitochondrial colocalization of IDH2(R172TAG)-GFP protein was confirmed using MitoTracker Red CMXRos. (i) GFP, (ii) MitoTracker, (iii) DIC, and (iv) merge of GFP and MitoTracker. Scale bar is 10 µm. Colocalization was also evident in neighboring cells (Supplementary Figure S1b).

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**Figure 2.** Metabolic and epigenetic effects of mutant IDH2 photoactivation, quantified by LC–MS/MS in HEK 293 cells stably expressing IDH2(R172TAG)-GFP and the PCKRS/tRNA<sub>CUA</sub> pair. (a) 2HG production is light-dependent. Metabolites were extracted 8 h after illumination at 365 nm (60 s, 9.5 mW/cm²). (b) Cellular 2HG accumulates following illumination of cells expressing IDH2(R172PCK)-GFP. The percent global level of 5-mC or 5-hmC of total cytosine, at each time point, is normalized to the corresponding value for nonilluminated sample. Error bars represent the standard deviation of the mean of at least three biological replicates.
communication will be interesting to investigate the immediate consequences of IDH mutation on the cellular pathways that these dioxygenases influence using the powerful approach we have reported. More broadly, we note that extensions of our approach will provide a strategy to rapidly and synchronously un cloak oncogenic mutations in diverse proteins in live cells, in culture or within animals. By coupling this strategy to increasingly powerful analytical methods, we anticipate that it will be possible to reveal the early events in transformation by diverse onco genes.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b07627.

**Supplementary Figures S1—S5 and methods and materials (PDF)**

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**Notes**

The authors declare no competing financial interest.

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