The traditional view is that receptor-initiated cell proliferation regulates metabolism indirectly.
Mammalian cells also depend on growth factor signaling for nutrient uptake.
Optimal cell proliferation requires two signals

Growth Signal

Fuel Signal
Most Oncogenes and Tumor Suppressors evolved to regulate cellular metabolism
Metabolites regulate signal transduction and gene expression

Metabolic Pathways
Nicholson, 2007

Pathways in Human Cancer
Weinberg, 2006
Tumorigenic mutations in metabolic enzymes do exist

- Succinate dehydrogenase – pheochromocytoma
- Fumarase – leiomyoma and leiomyosarcoma
- Mutations in these Krebs cycle enzymes are loss-of-function and recessive.
- Succinate / fumarate accumulation then indirectly activates the HIF-1α pathway.
Succinate accumulation as a result of SDH mutation inhibits proline hydroxylation of HIF-1
Effects of SDH inhibition on cellular redox state and succinate concentration

<table>
<thead>
<tr>
<th></th>
<th>scRNAi</th>
<th>Di3</th>
<th>Di4</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (nmol/mg)</td>
<td>44.0 ± 5.03</td>
<td>42.3 ± 9.76</td>
<td>46.3 ± 1.05</td>
</tr>
<tr>
<td>GSH + GSSG</td>
<td>45.9 ± 5.13</td>
<td>44.2 ± 9.80</td>
<td>48.7 ± 0.82</td>
</tr>
<tr>
<td>Succinate (μM)</td>
<td>120.6 ± 32.0</td>
<td>446.8 ± 86.1</td>
<td>440.9 ± 36.0</td>
</tr>
</tbody>
</table>

The levels of reduced (GSH) and total (GSH + GSSG)
Succinate blocks the ability of proline hydroxylase to hydroxylate HIF1
Dimethyl-succinate (DMS) treatment of cells induces HIF1
IDH mutations in glioblastoma

• Somatic missense mutations in NADP\(^+\)-linked isocitrate dehydrogenase (IDH) were observed in 12% of glioblastomas.

• It was reported that the IDH1 mutations observed result in loss of enzymatic function.

• All mutations were monoallelic and were limited to missense mutations of a single arginine residue in the enzyme’s active site.
IDH mutations are common in intermediate grade gliomas.

Yan, H., et al. NEJM 360:8, Feb 2009
IDH mutations impair the enzyme’s ability to oxidize isocitrate.

- But can a simple “loss of function” fully account for the effect of IDH mutations?
- Is IDH a tumor suppressor or an oncogene?

Yan, H., et al. NEJM 360:8, Feb 2009
Glioma-Derived Mutations in IDH1 Dominantly Inhibit IDH1 Catalytic Activity and Induce HIF-1α

Shimin Zhao,1,2 Yan Lin,1* Wei Xu,1,2* Wenqing Jiang,1,2* Zhengyu Zha,1 Pu Wang,1,2 Wei Yu,1,2 Zhiqiang Li,4 Lingling Gong,5 Yingjie Peng,6 Jianping Ding,6 Qunying Lei,1,3 Kun-Liang Guan,1,3,7† Yue Xiong1,2,8†

Normal cells

Tumor cells

IDH1

IDH1

α-KG

HIF-1α

PHD

HIF-1α

VEGF, GluT1, PGK1, etc.

Tumor development

α-KG

OH

OH

IDH1 and IDH2 mutations coordinate the same isocitrate carboxyl group.
Mutant IDH1 is not dominant negative

IDH activity assay

NADPH production
(Change in OD$_{340}$)

NADP$^+$  NADPH
isocitrate  $\rightarrow$ $\alpha$-ketoglutarate

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Reaction Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 µg WT</td>
<td>0.30</td>
</tr>
<tr>
<td>2 µg WT</td>
<td>0.25</td>
</tr>
<tr>
<td>2 µg WT + 2 µg R132H</td>
<td>0.20</td>
</tr>
<tr>
<td>4 µg vector</td>
<td>0.15</td>
</tr>
<tr>
<td>2 µg R132H</td>
<td>0.10</td>
</tr>
<tr>
<td>4 µg R132H</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Nature, 10 Dec 2009
Glioblastoma cells transfected with R132H IDH1 accumulate an abnormal metabolite.
The normal reverse IDH reaction: occurs in 2 steps

\[ \text{α-ketoglutarate} \rightarrow \text{isocitrate} \]

Step 1: carboxylation

\[ \text{NADPH} + \text{H}^+ \rightarrow \text{NADP}^+ \]

Step 2: reduction

Mutant IDH fails to carboxylate α-KG

2-hydroxyglutarate (2HG)
A heterodimer between wildtype and mutant IDH1 potentiates 2HG production.
2HG levels are elevated in human glioma samples with IDH1 mutations.

*Nature*, 10 Dec 2009
Glioma-derived IDH1 R132 mutations produce 2-hydroxyglutarate (2HG).

• Is 2HG merely a correlative byproduct of mutations at one specific arginine in IDH1?

• Does 2HG accumulate in other cancers?
Acute Myeloid Leukemias also display IDH1 mutations

Mardis et al., 2009
Screening human AML samples for elevated 2HG uncovers yet another IDH neomorph.
IDH2 R140 coordinates the same isocitrate carboxyl as IDH1 R132 and IDH2 R172
IDH neomorphs that produce 2HG may define a novel subset of AML.

<table>
<thead>
<tr>
<th>IDH status</th>
<th>≥3 cytogenetic abnormalities</th>
<th># Flt-3 mutant</th>
<th># NPM1 mutant</th>
<th># ASXL1 mutant*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type (n=60)</td>
<td>7/51</td>
<td>11/60</td>
<td>4/60</td>
<td>8/56</td>
</tr>
<tr>
<td>IDH2 mutant (R140Q n=7; R172K n=5)</td>
<td>0/12</td>
<td>0/11</td>
<td>0/9</td>
<td>0/10</td>
</tr>
</tbody>
</table>

*Samples with alterations which could not be confirmed to be somatic were excluded from analysis.*
What is mutant IDH doing in cancer?

• Approach:

  – Ask whether IDH mutations are particularly exclusive of any other common ongenic lesions.

  – This could suggest a common redundant function.
IDH1/2 neomorphic mutations are mutually exclusive with loss-of-functions TET2 mutations.

57 IDH1/2 mutant AMLs

28 TET2 mutant AMLs
TET2 is an α-ketoglutarate dependent dioxygenase that hydroxylates 5-methylcytosine.

\[
\begin{align*}
5\text{-MeC} & \rightarrow 5\text{-OH-MeC} \\
\alpha\text{-ketoglutarate} & \rightarrow \text{succinate} \\
\text{DNA demethylation}
\end{align*}
\]

Mutant IDH
IDH mutation inhibits hydroxylation of 5-methylcytosine by TET2.

Mock

TET2-FLAG

TET2-FLAG + IDH1 R132H

TET2-FLAG + IDH1 WT

Anti-5-OH-methylcytosine
Epigenetic modification in carcinogenesis

Transcriptional activators

Transcriptional repressors

$\alpha$KG $\rightarrow$ 2HG

Chen et al., Nat Rev Cancer 2010
Leukemia can be initiated by a block in differentiation

2HG

PML-RARα fusion protein

The production of abnormal retinoic acid receptors

Continuous repression of the RARα target genes

Blocked myeloid Differentiation at the promyelocyte stage

Acute Promyelocytic Leukemia (APL)

Myeloblast

Myelocyte

Metamyelocyte

Band Neutrophil

Segmented Neutrophil (PMN)

ATRA

ATRA-Induced Myelocytic Differentiation
Epigenetic modification in carcinogenesis

Transcriptional activators

Transcriptional repressors

$\alpha$KG $\rightarrow$ 2HG
Dynamics of histone lysine methylation

Histone methyltransferase

S-adenosyl-methionine → S-adenosyl-homocysteine

alpha-ketoglutarate

FAD

FADH₂

Succinate

LSD

Jumonji-C histone lysine demethylases

Me

[Diagram showing the process of histone lysine methylation with key enzymes and cofactors]
2HG is a competitive inhibitor of \( \alpha\)-KG in H3K9 demethylation
Gliomas exhibit enhanced levels of repressive H3K9 histone marks
IDH1 mutants induce progressive histone followed by DNA methylation.

<table>
<thead>
<tr>
<th>Passage</th>
<th>IDH1 WT</th>
<th>IDH1 R132H</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>7</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>12</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>17</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td>22</td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
</tr>
<tr>
<td>27</td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**Graphs:**

- **H3K9me3 levels**
  - IDH1 WT
  - IDH1 R132H

- **DNA Methylation levels**
  - IDH1 WT
  - IDH1 R132H
Histone methylation and cell differentiation

Adapted from Cloos et al, Genes Dev 2008
Primary Neural Progenitors Fail to Differentiate in culture when expressing 2HG-producing IDH mutants

![Graph showing the expression of β3-tubulin, GFAP, and p85 under different conditions of Retinoic Acid and IDH mutants.](image)
Histone/DNA demethylation are required for cell differentiation

Transcriptional activators

Transcriptional repressors

αKG  2HG
In gliomas, IDH mutation is also tightly associated with DNA hypermethylation

Glioma-CpG Island Methylator Phenotype (G-CIMP)

Noushmehr H et al, Cancer cell, 2010