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1. Introduction

Isocitrate dehydrogenases (IDHs) are enzymes long known to biologists as a component of the tricarboxylic acid (TCA) cycle that converts isocitrate to α-ketoglutarate (α-KG) with production of NADH and/or NADPH. However, it was mainly viewed as a “housekeeping” gene by cancer biologists with no previously defined role in cancer. This changed in 2008 with the discovery that IDH1 was frequently mutated in glioblastoma multiformes (GBMs) (Parsons et al., 2008). IDH mutations have now also been found in lower grade gliomas as well as in acute myelocytic leukemias. The purpose of this chapter is to review the normal functions of the IDH isoforms and their role in glioma brain tumors from initial discovery of a specific mutation in IDH1 to what is currently known about the mechanisms of action of mutant IDHs.

2. Normal function of isocitrate dehydrogenases

2.1 Isocitrate dehydrogenase enzymatic activity and structure

IDH is an enzyme whose activity is to oxidatively decarboxylate isocitrate producing α-ketoglutarate (α-KG) and CO₂ (Haselbeck & McAlister-Henn, 1993). A schematic of this reaction with all the family members is shown (Fig. 1). During this process, NAD⁺ or NADP⁺ is reduced to NADH or NADPH, respectively, depending on the isoform that is catalyzing this reaction. The IDH1 and IDH2 isoforms are NADP⁺ dependent and function as homodimers (Bailey & Colman, 1985; Kelly & Plaut, 1981). They are structurally related sharing approximately 70% sequence identity between the two isoforms (Xu et al., 2004). IDH1 is most highly expressed in liver while IDH2 show the greatest expression in muscle (Haselbeck et al., 1992; Jennings et al., 1994). However, both isoforms show moderate expression within a variety of other tissues including brain (Jennings et al., 1994). The IDH3 isoform is NAD⁺ dependent, functions as a heterotetramer consisting of 2 α, 1 β and 1 γ subunits (Ramachandran & Colman, 1980), and is structurally unrelated to IDH1 and IDH2 (Nichols et al., 1993; Nichols et al., 1995). IDH3 is the classical TCA cycle enzyme and plays an integral role in cellular energy metabolism. Consequently, IDH3 is found predominantly in the mitochondria (Haselbeck & McAlister-Henn, 1993). IDH2 also localizes to the mitochondria and has a mitochondrial signal peptide at its N-terminus (Nekrutenko et al., 1998). By contrast, IDH1 is localized mainly in the cytoplasm but has a type 1 peroxisomal targeting sequence permitting localization in peroxisomes (Henke et al., 1998; Nekrutenko et
al., 1998). The reactions catalyzed by IDH1/2 are reversible while the similar reaction catalyzed by IDH3 is irreversible. Because only IDH1 and IDH2 mutations have been shown to be important in malignancies, we will not be addressing IDH3 further.

Fig. 1. Enzymatic activity of wild type and mutant IDH isoforms.

Structural characteristics of mammalian IDH1 and IDH2 are well-known with elucidation of their crystal structures (Ceccarelli et al., 2002; Xu et al., 2004). A cartoon representation of the IDH1 homodimer is shown (IDH2 structure is similar) (Fig. 2). These enzymes dimerize with two active site in an open, inactive conformation, which is maintained by an intramolecular interaction between a conserved serine at position 94 and aspartic acid at position 279 in IDH1 blocking access to the active site. The IDH homodimer shifts to the closed, active conformation when this serine:aspartic acid interaction is lost permitting entry of an isocitrate:metal ion complex into the active site. The reaction proceeds with formation of $\alpha$-KG and NADPH which is released with either immediate reoccupation of the active site with another isocitrate:metal ion complex and NADP$^+$ or reassociation of the serine:aspartic acid interaction returning the enzyme back to an inactive state.

Fig. 2. Representation of wild type IDH1 homodimers shifting between the inactive and active conformations.

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2.2 Normal function of isocitrate dehydrogenase in cellular metabolism

Both IDH1 and IDH2 play key roles in various cellular metabolic functions. They are involved in the oxidation of polyunsaturated fatty acids within peroxisomes (IDH1) and mitochondria (IDH2) by using the NADPH generated by their enzymatic activity (Minard & McAlister-Henn, 1999; van Roermund et al., 1998). In the liver, IDH1 is regulated by sterol regulatory element-binding proteins and also generates NADPH for peroxisomal lipogenesis (Shechter et al., 2003). In pancreatic islet cells, IDH1 has an important role in cellular glucose sensing as evidenced by impairment of glucose-stimulated insulin secretion after knockdown of IDH1 expression (Ronnebaum et al., 2006). Consistent with these functions, IDH1 transgenic mice display fatty livers, hyperlipidemia, obesity and higher glucose sensitivity on glucose tolerance testing consistent with enhanced insulin secretion (Koh et al., 2004). Finally, IDH2 has probable roles in the TCA cycle as evidenced by the lack of pathology in most normal tissues of certain retinitis pigmentosa patients with a homozygous IDH3 subunit defect (Hartong et al., 2008). The reverse reaction (producing isocitrate and NADP\(^+\) from \(\alpha\)-KG and NADPH) by IDH2 has been proposed as a way of limiting flux through the TCA cycle and dissipating the proton electrochemical gradient across the inner mitochondrial membrane with heat generation (Sazanov & Jackson, 1994). These various metabolic functions that have been defined to date show the central role that IDH1 and IDH2 play at the crossroads of lipid synthesis and carbohydrate utilization.

2.3 The role of isocitrate dehydrogenase in response to oxidative stress

IDH1/2 also likely plays a part in the oxidative stress response and helps limit damage from such insults. Consistent with this idea, Mailloux et al. found that oxidative stress enhances \(\alpha\)-KG and NADPH production by IDH1/2 with a concomitant decrease in IDH3, \(\alpha\)-KG dehydrogenase, and succinate dehydrogenase activities decreasing utilization of the TCA cycle (Mailloux et al., 2007). NADPH produced by IDH1/2 can also be used by glutathione reductase for converting the oxidized form of glutathione (GSSG) to the reduced form (GSH) that can neutralize free radicals and reactive oxygen species (Jo et al., 2001; Kehrer & Lund, 1994; Lee et al., 2002). While the pentose phosphate pathway is the major source of NADPH required for regeneration of GSH, IDH1/2 can also contribute to this NADPH pool (Winkler et al., 1986). In addition, Lee et al. were able to generate a series of NIH3T3 derivatives that expressed varying levels of IDH1 and found that the ratio of GSH:GSSG was directly correlated with IDH1 expression level consistent with a role of this enzyme in the regeneration of GSH (Lee et al., 2002). Reduced expression of both IDH1 and IDH2 results in higher levels of reactive oxygen species and greater oxidative damage in response to an oxidative insult (Jo et al., 2001; Lee et al., 2002). In fact, numerous reports have now demonstrated that overexpression of IDH1 and IDH2 can protect cells against a variety of insults that produce oxidative stress (Jo et al., 2002; Kim et al., 2007; Lee et al., 2004; Shin et al., 2004). Based on this wealth of evidence demonstrating a role in the response of IDH1/2 to oxidative damage, the NADP\(^+\)-dependent IDHs clearly have significant functions beyond energy metabolism and biosynthetic processes.

3. Isocitrate dehydrogenase mutations in glial and other neoplasms

3.1 Discovery of isocitrate dehydrogenase 1 mutations in glioblastomas

GBMs are highly aggressive brain tumors classified by the World Health Organization (WHO) grading system as grade IV astrocytomas (Louis et al., 2007). While outcomes for
patients with this diagnosis have gradually improved with better surgical/radiation therapy techniques and temozolomide chemotherapy, median survival still remain only slightly longer than one year (Stupp et al., 2005). With recent genomic technology advances, projects were initiated to perform detailed genomic analysis of various malignancies including GBMs. This effort quickly bore fruit with the discovery by Parson et al. that IDH1 is frequently mutated in GBMs (Parsons et al., 2008). They performed an initial screen consisting of comprehensive analysis of 20,661 protein-coding genes in 22 GBM samples. This yielded 21 mutated genes that were further analyzed in a followup screen on 83 additional GBMs. In addition to finding expected mutations at several genes known to be important in GBMs, the IDH1 gene was surprisingly found to be altered in 11% of analyzed GBMs. Most striking, mutations were invariably at a highly conserved arginine at position 132 (R132) found in the isocitrate binding site and was mutated to either histidine (R132H) (10 of 12) or serine (R132S) (remaining 2). This unexpected finding implicated IDH1 in the development and/or maintenance of glial neoplasms.

Followup studies confirmed the presence of IDH mutations in GBMs (Balss et al., 2008; Hartmann et al., 2009; Sanson et al., 2009; Watanabe et al., 2009; Yan et al., 2009) (Table 1). GBMs are classified as primary (de novo) or secondary depending on whether they arise spontaneously or from malignant transformation of a low grade glioma. Interestingly, the frequency of IDH1 mutations was significantly higher in secondary (82%) than primary (6%) GBMs. Thus, the incidence of IDH mutations in an undifferentiated cohort of GBMs would depend on the distribution of primary and secondary tumors in that group. Of note, initial studies suggest that pediatric GBMs only rarely harbor IDH mutations (Antonelli et al., 2010; Balss et al., 2008; Paugh et al., 2010; Yan et al., 2009). However, a recent paper suggests that pediatric malignant glioma patients ≥ 14 years of age appear to harbor IDH mutations at a substantial rate (7 of 20) (Pollack et al., 2011). This result suggests that high-grade

<table>
<thead>
<tr>
<th>WHO Grade</th>
<th>Tumor type</th>
<th>Balss/Hartmann</th>
<th>Yan</th>
<th>Watanabe</th>
<th>Sanson</th>
<th>Total (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV 1&lt;sup&gt;st&lt;/sup&gt; GBM</td>
<td>7/99</td>
<td>6/123</td>
<td>3/59</td>
<td>11/183</td>
<td>27/464 (5.8%)</td>
<td></td>
</tr>
<tr>
<td>IV 2&lt;sup&gt;nd&lt;/sup&gt; GBM</td>
<td>7/8</td>
<td>11/13</td>
<td>28/34</td>
<td>10/13</td>
<td>56/68 (82.4%)</td>
<td></td>
</tr>
<tr>
<td>III AA</td>
<td>148/228</td>
<td>38/52</td>
<td>21/27</td>
<td>9/18</td>
<td>216/325 (66.5%)</td>
<td></td>
</tr>
<tr>
<td>III AOA</td>
<td>128/177</td>
<td>7/7</td>
<td>10/14</td>
<td>34/54</td>
<td>179/252 (71.0%)</td>
<td></td>
</tr>
<tr>
<td>III AO</td>
<td>130/174</td>
<td>34/36</td>
<td>6/8</td>
<td>24/49</td>
<td>194/267 (72.7%)</td>
<td></td>
</tr>
<tr>
<td>II A</td>
<td>167/227</td>
<td>27/30</td>
<td>60/68</td>
<td>10/12</td>
<td>264/337 (78.3%)</td>
<td></td>
</tr>
<tr>
<td>II OA</td>
<td>63/76</td>
<td>3/3</td>
<td>16/17</td>
<td>26/34</td>
<td>108/130 (83.1%)</td>
<td></td>
</tr>
<tr>
<td>II O</td>
<td>111/128</td>
<td>43/51</td>
<td>31/39</td>
<td>41/54</td>
<td>226/272 (83.1%)</td>
<td></td>
</tr>
<tr>
<td>I Pilocytic Astrocytoma</td>
<td>1/41</td>
<td>0/21</td>
<td>3/31</td>
<td>NT</td>
<td>4/93 (4.3%)</td>
<td></td>
</tr>
<tr>
<td>I-III Ependymoma</td>
<td>0/31</td>
<td>0/30</td>
<td>0/24</td>
<td>NT</td>
<td>0/85 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> Combined results of Balss and Hartmann studies due to duplication of some cases (Balss et al., 2008; Hartmann et al., 2009). <sup>2</sup> (Yan et al., 2009). <sup>3</sup> (Watanabe et al., 2009). <sup>4</sup> (Sanson et al., 2009).

Abbreviations: WHO, World Health Organization; GBM, glioblastoma multiforme; AA, anaplastic astrocytoma; AOA, anaplastic oligoastrocytoma; AO, anaplastic oligodendroglioma; A, astrocytoma; OA, oligoastrocytoma; O, oligodendrogloma; NT, not tested.

Table 1. Frequency of IDH mutations in various glial brain tumors.
gliomas in younger pediatric patients may be a different entity than those presenting in late adolescence with the older pediatric patients likely having a tumor that may be more similar to such tumors that present in young adulthood (eg. 20-40 years of age).

3.2 Isocitrate dehydrogenase mutations in other gliomas

Since secondary GBMs have a high incidence of IDH mutations, these mutations were postulated to be present in low grade gliomas as well. As predicted, pooled results found IDH mutations in 65% to 80% of grade II/III astrocytomas (Table 1) (Balss et al., 2008; Hartmann et al., 2009; Sanson et al., 2009; Watanabe et al., 2009; Yan et al., 2009). Grade II/III oligodendrogliomas and oligoastrocytomas also had a high incidence of IDH1 mutations in the 70-85% range. Finally, pilocytic astrocytomas only rarely harbor IDH1 mutations (<5%) while no IDH1 mutations were found in ependymomas of any grade (Balss et al., 2008; Watanabe et al., 2009; Yan et al., 2009).

Although IDH1 and IDH2 reside largely in different subcellular compartments, they have the same enzymatic activity, utilize NADP⁺, and are believed to provide some redundant function(s) in the cell. Therefore, Yan et al. also sequenced IDH2 in addition to IDH1 in their series of brain tumors and found that IDH2 was, in fact, mutated at a low frequency on arginine at position 172 (R172), the comparable residue to R132 of IDH1 (Yan et al., 2009). Based on pooled results from multiple studies examining glioma brain tumors, when IDH is mutated, IDH1 is affected 96% of the time and IDH2 is affected in only 4% of cases (Table 2) (Hartmann et al., 2009; Sonoda et al., 2009; Yan et al., 2009). In addition, mutation on one IDH isoform was always mutually exclusive for mutation on the other isoform.

<table>
<thead>
<tr>
<th>Type</th>
<th>Balss¹</th>
<th>Yan²</th>
<th>Hartman³</th>
<th>Sanson⁴</th>
<th>Sonoda⁵</th>
<th>Total (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDH1</td>
<td>221</td>
<td>161</td>
<td>716</td>
<td>155</td>
<td>39</td>
<td>1292</td>
</tr>
<tr>
<td>R132H</td>
<td>205</td>
<td>142</td>
<td>664</td>
<td>138</td>
<td>39</td>
<td>1188 (92.0%)</td>
</tr>
<tr>
<td>R132C</td>
<td>8</td>
<td>7</td>
<td>29</td>
<td>5</td>
<td>--</td>
<td>49 (3.8%)</td>
</tr>
<tr>
<td>R132L</td>
<td>1</td>
<td>7</td>
<td>2</td>
<td>2</td>
<td>--</td>
<td>12 (0.9%)</td>
</tr>
<tr>
<td>R132S</td>
<td>4</td>
<td>7</td>
<td>11</td>
<td>3</td>
<td>--</td>
<td>22 (1.7%)</td>
</tr>
<tr>
<td>R132G</td>
<td>2</td>
<td>1</td>
<td>10</td>
<td>7</td>
<td>--</td>
<td>20 (1.5%)</td>
</tr>
<tr>
<td>R132V</td>
<td>1</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1 (0.1%)</td>
</tr>
<tr>
<td>IDH2</td>
<td>NT</td>
<td>9</td>
<td>31</td>
<td>NT</td>
<td>1</td>
<td>41</td>
</tr>
<tr>
<td>R172K</td>
<td>NT</td>
<td>4</td>
<td>20</td>
<td>NT</td>
<td>1</td>
<td>25 (61.0%)</td>
</tr>
<tr>
<td>R172M</td>
<td>NT</td>
<td>3</td>
<td>6</td>
<td>NT</td>
<td>--</td>
<td>9 (22.0%)</td>
</tr>
<tr>
<td>R172G</td>
<td>NT</td>
<td>2</td>
<td>--</td>
<td>NT</td>
<td>--</td>
<td>2 (4.9%)</td>
</tr>
<tr>
<td>R172W</td>
<td>NT</td>
<td>--</td>
<td>5</td>
<td>NT</td>
<td>--</td>
<td>5 (12.2%)</td>
</tr>
</tbody>
</table>

¹ (Balss et al., 2008), ² (Yan et al., 2009), ³ (Hartmann et al., 2009), ⁴ (Sanson et al., 2009), ⁵ (Sonoda et al., 2009).

Abbreviations: IDH, isocitrate dehydrogenase; NT, not tested.

Table 2. Type and frequency of IDH1/IDH2 mutations in gliomas.

The frequency distribution of mutations in IDH1/2 is summarized on Table 2 (Balss et al., 2008; Hartmann et al., 2009; Sanson et al., 2009; Sonoda et al., 2009; Yan et al., 2009). The great majority of mutations at R132 in IDH1 and R172 in IDH2 result from single nucleotide changes within the codon. Possible changes at these codons are shown with the frequency they are observed (Fig. 3). It is interesting to note that nearly 92% of IDH1 mutations were to
histidine. Thus, a very significant selective advantage must be at play for this particular point mutation. There may also be slight selection for alterations to cysteine with an increased incidence of between 2-4 fold higher than any of the other three observed amino acids although total number of cases are relatively small. Numbers for IDH2 mutations are also relatively small, but does suggest skewing towards mutation of R172 to lysine (R172K).

<table>
<thead>
<tr>
<th>IDH1 type</th>
<th>Codon</th>
<th>AA</th>
<th>Frequency of mutation</th>
<th>IDH2 type</th>
<th>Codon</th>
<th>AA</th>
<th>Frequency of mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>wildtype IDH1</td>
<td>C&gt;G&gt;T</td>
<td>Arg</td>
<td>N/A</td>
<td>wildtype IDH2</td>
<td>A&gt;G&gt;C</td>
<td>Arg</td>
<td>N/A</td>
</tr>
<tr>
<td>R132S</td>
<td>A&gt;G&gt;T</td>
<td>Ser</td>
<td>1.7%</td>
<td>conserved</td>
<td>C&gt;G&gt;C</td>
<td>Arg</td>
<td>N/A</td>
</tr>
<tr>
<td>R132G</td>
<td>C&gt;G&gt;T</td>
<td>Gly</td>
<td>1.5%</td>
<td>R172G</td>
<td>A&gt;G&gt;C</td>
<td>Gly</td>
<td>4.8%</td>
</tr>
<tr>
<td>R132C</td>
<td>T&gt;G&gt;T</td>
<td>Cys</td>
<td>3.8%</td>
<td>R172W</td>
<td>A&gt;G&gt;C</td>
<td>Trp</td>
<td>12.2%</td>
</tr>
<tr>
<td>R132H</td>
<td>A&gt;G&gt;T</td>
<td>His</td>
<td>92.0%</td>
<td>R172K</td>
<td>A&gt;G&gt;C</td>
<td>Lys</td>
<td>61.0%</td>
</tr>
<tr>
<td>not seen</td>
<td>C&gt;G&gt;T</td>
<td>Pro</td>
<td>0%</td>
<td>not seen</td>
<td>A&gt;G&gt;C</td>
<td>Thr</td>
<td>0%</td>
</tr>
<tr>
<td>R132L</td>
<td>C&gt;T&gt;T</td>
<td>Leu</td>
<td>0.9%</td>
<td>R172M</td>
<td>A&gt;G&gt;C</td>
<td>Met</td>
<td>22.0%</td>
</tr>
<tr>
<td>conserved</td>
<td>C&gt;G&gt;A</td>
<td>Arg</td>
<td>N/A</td>
<td>conserved</td>
<td>A&gt;G&gt;A</td>
<td>Arg</td>
<td>N/A</td>
</tr>
<tr>
<td>conserved</td>
<td>C&gt;G&gt;A</td>
<td>Arg</td>
<td>N/A</td>
<td>not seen</td>
<td>A&gt;G&gt;C</td>
<td>Ser</td>
<td>0%</td>
</tr>
<tr>
<td>conserved</td>
<td>C&gt;G&gt;A</td>
<td>Arg</td>
<td>N/A</td>
<td>not seen</td>
<td>A&gt;G&gt;T</td>
<td>Ser</td>
<td>9%</td>
</tr>
<tr>
<td>R132V</td>
<td>C&gt;T&gt;T</td>
<td>Val</td>
<td>0.1%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3. Possible mutations in codon 132 of IDH1 and codon 172 of IDH2 with observed frequency in glial neoplasms.

### 3.3 Isocitrate dehydrogenase mutations in other malignancies

After discovery of the specific IDH1 and IDH2 mutations in gliomas, there was significant interest to determine whether these mutations were also present in other malignancies. Despite screening of a large number of tumor specimens, very few non-glioma, solid malignancies were found to contain these mutations. One study found 2 of 75 (2.7%) prostate cancers with IDH1 mutations while a second study found a metastatic melanoma with an IDH1 mutant (Bleeker et al., 2009; Kang et al., 2009; Lopez et al., 2010). Kang et al. also found an IDH1 mutation in 1 of 60 cases of B-cell acute lymphoblastic leukemia (Kang et al., 2009). Mutations at both IDH1 and IDH2 have now been shown to be present in acute myelogenous leukemias (AMLs). Mardis et al. were the first to report that 16 of 188 (8.5%) primary, cytogenetically normal AMLs had mutation at R132 in IDH1 (Mardis et al., 2009). Likewise, Ward et al. found in their cohort that 6 of 60 (10%) karyotypically normal AMLs had the expected IDH1 mutation (Ward et al., 2010). They also found that these AMLs were actually more likely to harbor mutations in IDH2 with the previously defined R172K mutation seen in 5 cases and a new mutation altering arginine at position 140 to glutamine (R140Q). These specific mutations all resulted in elevated levels of 2-hydroxyglutarate (2-HG), a marker of mutant IDH activity (details of this enzymatic activity will be covered in section 4.3). Marcucci et al. reported similar results in a Cancer and Leukemia Group B study where out of 358 cases of cytogenetically normal AMLs, 47 had IDH1 mutations at R132, 13 had IDH2 mutations at R172 and 56 had IDH2 mutations at R140 (Marcucci et al., 2010). Based on these studies, it appears that ~30% of cytogenetically normal AMLs harbor mutations in IDH1/2. Also, whereas the great majority of IDH mutations found in gliomas involve IDH1, mutations of IDH in AML are more evenly distributed between IDH1 and IDH2 with a slight favoring of the latter. Unlike the case where IDH1 and IDH2 mutations were always mutually exclusive, rare instances of AMLs with both mutation present has been reported (Paschka et al., 2010).
3.4 Clinical outcomes in gliomas harboring isocitrate dehydrogenase mutations

With the identification of IDH1 mutations in GBMs, Parsons et al. made an intriguing observation that patients with this mutation had a significantly better survival (Parsons et al., 2008). However, since their clinical series only included 11 patients with IDH1 mutations, this finding required validation. At this time, multiple reports have confirmed that IDH1/2 mutations predict for improved outcomes in patients with GBMs as well as lower grade glial neoplasms (Dubbink et al., 2009; Nobusawa et al., 2009; Sanson et al., 2009; Sonoda et al., 2009; Yan et al., 2009). A summary of these reports is shown on Table 3.

<table>
<thead>
<tr>
<th>Publications</th>
<th>Glioma type</th>
<th>wtIDH</th>
<th>mutIDH</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parsons²</td>
<td>GBM</td>
<td>1.1 yrs (79)</td>
<td>3.8 yrs (11)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Yan³</td>
<td>GBM</td>
<td>15 (115)</td>
<td>31 (14)</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>20 (14)</td>
<td>65 (38)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sanson⁴</td>
<td>GBM, 1⁰</td>
<td>14 (172)</td>
<td>27.4 (11)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Grade 3</td>
<td>19.4 (54)</td>
<td>81.1 (67)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Grade 2</td>
<td>60.1 (23)</td>
<td>150.9 (77)</td>
<td>0.01</td>
</tr>
<tr>
<td>Nobusawa⁵</td>
<td>GBM, all</td>
<td>9.9 (186)</td>
<td>24.0 (17)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>GBM, 1⁰</td>
<td>10.0 (185)</td>
<td>31.6 (9)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Sonoda⁶</td>
<td>GBM (1⁰ and 2⁰)</td>
<td>17 (57)</td>
<td>66 (5)</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>22 (8)</td>
<td>50 (13)</td>
<td>0.001</td>
</tr>
<tr>
<td>Dubbink⁷</td>
<td>Grade 2</td>
<td>48 (7)</td>
<td>98 (42)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

* in months except when otherwise indicated, ² (Parsons et al., 2008), ³ (Yan et al., 2009), ⁴ (Sanson et al., 2009), ⁵ (Nobusawa et al., 2009), ⁶ (Sonoda et al., 2009), ⁷ (Dubbink et al., 2009).

Abbreviations: OS, overall survival; wtIDH, wild type isocitrate dehydrogenase; mutIDH, mutant isocitrate dehydrogenase; GBM, glioblastoma multiforme; AA, anaplastic astrocytomas; yrs, years.

3.5 Factors in gliomas correlating with isocitrate dehydrogenase mutations

Certain clinical characteristics and markers have been associated with the presence of IDH1 mutations in GBMs. Patients with tumors harboring this mutation tend to be younger with an average age in the 40-50 year range versus 60+ years in patients lacking this mutation (Nobusawa et al., 2009). Although IDH1 mutations were associated with a younger age, presence of this mutation was still independently prognostic. Patients with tumors containing IDH1 mutations were also more likely to have a longer duration of symptoms consistent with a slower growing, less aggressive tumor. In addition, the presence of IDH1 mutations appears to be associated with certain genetic abnormalities including TP53 mutation, PTEN mutation, lack of EGFR amplification, loss of heterozygosity on 19q, and loss of CDKN2A/B (Nobusawa et al., 2009; Yan et al., 2009). These correlations are not surprising given the predominance of IDH1 mutation in secondary GBMs. However, even when only primary GBMs are considered, correlation between IDH mutations and TP53 mutation/lack of EGFR amplification was maintained (Nobusawa et al., 2009).
3.6 Isocitrate dehydrogenase mutations associate with proneural glioblastomas

Expression profiling of a series of high-grade astrocytomas have permitted subclassification based on a molecular signature. Phillips et al. performed one such analysis whereby 115 grade 3 and 4 astrocytomas were evaluated (Phillips et al., 2006). Based on their analysis, 3 distinct subgroups termed proneural, proliferative and mesenchymal could be identified based on similarities in gene expression pattern. The proneural group had markedly better prognosis and expressed genes associated with normal brain and neurogenesis. The proliferative group had a poor prognosis and expressed genes in a pattern that resembled highly proliferative cell lines. The mesenchymal group likewise had a poor prognosis and expressed genes that resembled tissues of mesenchymal origin. Since this report, the National Institutes of Health (NIH) established The Cancer Genome Atlas (TCGA) program that was charged with generating comprehensive multi-dimensional maps of the key genomic changes in major types of cancers. GBM was chosen as the pilot disease for this program. The expression profiling of GBMs for TCGA also permitted classification into subtypes (proneural, neural, classical, mesenchymal) similar to that described by Phillips et al. (Fig. 4) (Phillips et al., 2006; Verhaak et al., 2010). Interestingly, IDH1 was mutated in 11 of 37 cases (30%) of proneural GBMs with only a single case of mutant IDH1 found outside of the proneural group with that case being classified in the neural group (Verhaak et al., 2010). This analysis also validated an association between mutations in TP53 and the presence of IDH1 mutations that had previously been noted. A subsequent updated TCGA report continues to show a tight association between IDH1 mutation and this favorable GBM subtype (Noushmehr et al., 2010).

4. Functional consequence of isocitrate dehydrogenase mutations

4.1 Isocitrate dehydrogenase mutants lose normal enzymatic activity

Based on modeling studies, the side chain of R132 can form three hydrogen bonds with the α- and β-carboxyl groups of isocitrate while other residues within the binding site forms no more than two such bonds (Zhao et al., 2009). Thus, replacement of R132 is likely to impair
interactions between isocitrate and this site. In fact, this residue in IDH1 has been previously mutated to glutamic acid (R132E) and resulted in almost complete abrogation of enzymatic activity (Jennings et al., 1997). Zhao et al. show that the activity of the R132H, R132C and R132S mutants dropped to less than 20% of the wild type IDH1 enzyme with a corresponding increase in the Km for isocitrate by 60 to 94-fold (Zhao et al., 2009). Porcine IDH2 have also been mutated at the site comparable to R172 in human IDH2 (R133Q) and found to have decreased activity and increased Km for isocitrate (Soundar et al., 2000). All of the mutations found in IDH1 at R132 and in IDH2 at R172 have now been tested and the normal enzymatic activity is impaired in each case (Ichimura et al., 2009; Zhao et al., 2009).

4.2 Possible dominant negative activity of mutant isocitrate dehydrogenase 1
Because R132 mutations were so specific and alterations in the second allele had not been seen, this had the profile of an activating mutation. This hypothesis appeared to be incorrect with the discovery that R132 mutations resulted in loss of enzymatic activity (see section 4.1) (Yan et al., 2009; Zhao et al., 2009). However, since homozygous deletions or other inactivating mutations of IDH1/2 had not been reported, the IDH1 R132 mutations (and the IDH2R172 mutations) were clearly acting in a more complex fashion. This was explained to some extent with the report that R132 IDH1 mutants can act as a dominant negative inhibitor (Zhao et al., 2009). To show this, His-tagged wild type IDH1 and FLAG-tagged R132H mutant were purified, mixed and subjected to affinity purification using nickel resin and anti-FLAG beads to obtain preparations of wild type homodimer, R132H homodimers and wild type:R132H heterodimers. The R132H homodimer was found to have no activity while the wild type:R132 heterodimer had 4% of the wild type homodimer activity. While this result partially explained how mutant IDH1 can act as a tumor suppressor in the absence of deletion of its second allele, this model was still not completely satisfying. In this model, mutant IDH1 needs to be in great excess (although amplification/overexpression has never been shown) or the wild type:mutant IDH1 interactions needs to be favored over wild type:wild type interactions (although such differential affinity has also never been demonstrated). In addition, the cellular metabolite profiles of cells engineered either to express the R132H mutant or to suppress expression of wild type IDH1 have been assessed and showed very little similarity again consistent with the putative dominant negative function of mutant IDH1 having little if any role in vivo (Reitman et al., 2011). Finally, Jin et al. found that the various IDH mutants did not associate with or inhibit the activity of the corresponding native IDH enzyme (Jin et al., 2011).

4.3 Isocitrate dehydrogenase mutations results in a neomorphic enzyme activity
Based on discussion in the previous section, if these mutations at R132 in IDH1 (or R172 in IDH2) were activating, it would better fit the available observations. Complicating this assertion, though, was the fact that the normal enzymatic activity of IDH1 was severely hampered when R132 was mutated. This hypothesis was finally proven with the report that 2-hydroxyglutarate (2-HG) accumulates in glioma cells that express the R132H mutant (Dang et al., 2010). In particular, the (R) enantiomer of 2-HG (D-2-hydroxyglutarate, D-2-HG) was detected with no (S) enantiomer (L-2-hydroxyglutarate, L-2-HG) found in mutant IDH1-expressing cells. Like previous investigators, Dang et al. found that the R132H mutant lost the ability to oxidative decarboxylate isocitrate to α−KG (Dang et al., 2010; Yan et al., 2009; Zhao et al., 2009). However, this mutant now gained a new activity, namely, the

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NADPH-dependent reduction of α-KG to 2-HG (Fig. 1) (Dang et al., 2010). Importantly, coexpression of wild type IDH1 and the R132H mutant did not reduce this new enzymatic activity but actually appear to enhance it. This has led to the suggestion that heterodimers can more efficiently produce 2-HG due to higher local concentrations of α-KG and NADPH produced by the wild type partner (Fig. 5). The x-ray structures of wild type IDH1 and the R132H mutant have been compared (Dang et al., 2010; Xu et al., 2004). Overall, R132 acts as a gate-keeper residue orchestrating the hinge movement between an open and closed conformation with the histidine mutation favoring a shift to the closed conformation which may increase binding of a new substrate (α-KG) and cofactor (NADPH) (Fig. 5).

Ward et al. performed a comparable analysis of the R172K IDH2 mutant found in AMLs and gliomas (Ward et al., 2010). Similar to the finding of Dang et al., this IDH2 mutant also displayed α-KG-dependent NADPH consumption and accumulation of 2-HG within expressing cells and surrounding media (Dang et al., 2010; Ward et al., 2010). Interestingly, they also found that knockdown of both wild type IDH1 and IDH2 with siRNA dramatically decreased proliferative capacity of a cancer cell line (Ward et al., 2010). These findings provide another explanation for why the corresponding normal IDH allele is not deleted and actually appears to always be present with the mutant IDH allele. Finally, this study found a new mutant IDH2 (R140Q) that also resulted in accumulation of 2-HG in cytogenetically normal AMLs. R140 is also a highly conserved residue and structural modeling puts it immediately adjacent to R172 in the isocitrate binding site, which helps explain acquisition of this neomorphic enzymatic activity in R140 mutants. To date, R140 mutations in IDH2 have not been identified in gliomas. Based on previous discussions, IDH1 mutations at R132 and IDH2 mutations at R140 and R172 gain similar new enzymatic function. However, differences must still exist between these mutations due to the observed disparity in distribution of IDH1 and IDH2 mutations in gliomas (ratio of ~9:1) and AMLs (ratio of ~2:3). One factor for this difference may lie in their respective subcellular location. IDH1 is primarily cytosolic and peroxisomal where NADPH is more limiting while IDH2 is mitochondrial where NADPH is more readily available because it can be easily interchanged with NADH produced by IDH3 through the action of H\(^+\)-transhydrogenase (Sazanov & Jackson, 1994). Despite this, both mutant IDH1 and IDH2 can produce 2-HG,
which can readily pass throughout the cell and even be secreted, and no definite functional
differences between the two mutant enzymes have been demonstrated.

5. The effect of mutant isocitrate dehydrogenase on normal cellular functions

5.1 The effect of mutant isocitrate dehydrogenase on cellular metabolism

Mutant IDHs are clearly selected for in gliomas and AMLs. While wild type IDH1/2 have
well-defined roles in metabolism, it remains unclear in what way expression of mutant
IDHs will alter these various processes. One consequence of mutant IDH expression is
decreased NADPH levels, which can potentially affect cellular biosynthetic processes such
as lipogenesis. Similarly, depletion of cytosolic NADPH may be sensed in the cell as a low
nutrient status leading to a response marked by increasing cellular nutrient consumption
(eg. increase glucose transporters, increase throughput in the pentose phosphate pathway,
etc.). These responses may provide malignant cells expressing mutant IDHs with a selective
growth advantage. Reitman et al. have now profiled >200 metabolites in human
oligodendroglioma cells engineered to express mutant IDH1 and IDH2 (Reitman et al.,
2011). One striking finding from this study was that the profile of mutant IDH-expressing
cells were very similar to those of corresponding cells treated with octyl-2-HG, a cell
permeable precursor of 2-HG. This directly implicates this mutant IDH product as a key
component of the cellular changes seen in mutant IDH-expressing cells. The main classes of
changes found on this study include increases in free amino acids, increases in lipid
precursors such as glycerol-phosphates and glycerophosphocholine, depletion of TCA cycle
intermediaries and depletion of N-acetylated amino acids such as N-acetyl-aspartate and N-
acetyl-aspartyl-glutamate, two of the most abundant compounds found in brain. Such
widespread changes are likely a consequence of global changes in gene expression that will
be discussed in sections 5.3-5.4 on epigenetic changes associated with mutant IDHs.

5.2 Mutant isocitrate dehydrogenase and hypoxia signaling

In addition to its effects on cellular metabolism, mutant IDHs may alter hypoxic response.
Zhao et al. showed that expression of the R132H mutant in U87MG and 293T cells induced
HIF-1α expression in a response that could be mitigated by treatment of cells with octyl-α-
KG, a cell-permeable derivative of α-KG (Zhao et al., 2009). Prolyl hydroxylase utilizes α-KG
to hydroxylate conserved prolines on HIF-1α leading to ubiquitination and subsequent
rapid degradation (for review, see (Semenza, 2007)). Expression of the R132H mutant can
theoretically deplete α-KG that is needed for HIF-1α proline hydroxylation or, alternatively,
the 2-HG product of R132H may act as a competitive inhibitor of prolyl hydroxylase
limiting HIF-1α proline hydroxylation. Since α-KG levels in mutant IDH1-expressing cells
generally change very little while 2-HG levels rise tremendously (Dang et al., 2010), the
latter hypothesis was more likely to be correct. Direct evidence for this have now been
reported by Xu et al. whereby treatment of cells with octyl-2-HG induces HIF-1α in a
process that is reversible with concomitant treatment with octyl-α-KG (Xu et al., 2011). The
consequence of HIF-1α stabilization is induction of HIF-dependent genes such as vascular
endothelial growth factor (VEGF), a pro-angiogenic factor, and glucose transporter 1 (glut1),
which can alter metabolism and nutrient consumption (Zhao et al., 2009). While this HIF-1α
response and its consequence is certainly intriguing, it still remains unclear how much of a
role this induction actually plays in the tumorigenic process. HIF-1α and VEGF induction
may contribute to the angiogenic phenotype seen in GBMs (for review, see (Van Meir et al., 2010)). However, since IDH mutation is a positive prognostic factor, that would suggest that HIF-1α induction is not a major function of IDH mutants (Nobusawa et al., 2009; Parsons et al., 2008; Yan et al., 2009). This is further supported by the lack of significant tumor angiogenesis in grade II and III gliomas of which >70% contain IDH mutations and the lack of HIF-1 target gene induction in AMLs with IDH mutation (Balss et al., 2008; Hartmann et al., 2009; Mardis et al., 2009; Yan et al., 2009).

5.3 Mutant isocitrate dehydrogenase is associated with a hypermethylation phenotype in gliomas

Association of IDH1 mutations with the proneural GBM subtype was a key finding from the assessment of GBMs in TCGA based mainly on expression profiling (Verhaak et al., 2010). Since that project acquired a host of genetic and epigenetic information about GBMs, investigators also looked for other factors that may associate with mutant IDH1. This culminated in work that found a glioma-CpG island methylator phenotype (G-CIMP) that defined a subgroup of GBMs that was tightly associated with IDH1 mutations (Noushmehr et al., 2010). Using the Illumina GoldenGate and/or Infinium methylation array platform, 272 TCGA GBM samples were assessed. Hypermethylation in a subset of loci was seen in 24 samples from this group that was reminiscent of the CpG island methylator phenotype (CIMP) previously described in colorectal cancer (Noushmehr et al., 2010; Toyota et al., 1999). 21 of 24 G-CIMP samples were classified as a proneural subtype and this represented approximately 30% of all proneural tumors. A subset of this cohort (207 samples) was then assessed for IDH mutations with the discovery of 18 IDH1 and no IDH2 mutations. Interestingly, all 18 tumors that had IDH1 mutations were labeled G-CIMP+ (18/23). Patients with proneural GBMs have been previously shown to have a better prognosis (Phillips et al., 2006; Verhaak et al., 2010). This study replicated that finding but found that G-CIMP positivity was an even stronger predictor of better outcomes (Noushmehr et al., 2010). Patients with G-CIMP+ proneural tumors (n=20) had a median survival of ~4 years while patients with G-CIMP- proneural tumors (n=49) did no better than patients with other subtypes (n=184) (median survivals of ~1 year). The association between mutant IDH1 and G-CIMP positivity also held in grade II and III gliomas and G-CIMP positivity still predicted for survival in the lower grade tumors. These results have now been replicated in an independent set of gliomas with the presence of IDH mutations correlating more strongly with hypermethylation than TP53 mutation or lack of EGFR alterations (Christensen et al., 2011). Hypermethylation of the O-6-methylguanine-DNA-methyltransferase (MGMT) promoter is highly associated with better outcomes in patients with GBM (Hegi et al., 2005; Stupp et al., 2009). Given its prognostic value, MGMT methylation testing has become relatively standard in the pathologic workup of GBMs. Although MGMT was not among the 50 most differentially hypermethylated genes on the TCGA study (Noushmehr et al., 2010), MGMT hypermethylation is correlated with IDH1 mutations in gliomas (Christensen et al., 2011; Laffaire et al., 2010; Sanson et al., 2009).

5.4 Mutant isocitrate dehydrogenase is associated with hypermethylation in AMLs

Previously, AMLs without known genetic or molecular features were found to be classifiable into five distinct clusters with particular epigenetic signatures (Figueroa et al., 2010b). Similar to the association of IDH1 mutation and G-CIMP, IDH1/2 mutations were
also associated with certain DNA methylation patterns in AML (Figueroa et al., 2010a). In fact, AMLs with IDH mutations fell primarily in two epigenetically-defined clusters that tended toward increased DNA methylation, which was reminiscent of G-CIMP (Figueroa et al., 2010a; Noushmehr et al., 2010). Hypermethylation was associated with decreased expression of the relevant gene in the majority of cases. Unlike GBMs where >90% of IDH mutations were on IDH1, mutations in AMLs are more equally distributed permitting comparisons in the type of methylation phenotypes that arise. Despite the IDH isoforms localizing to different subregions of the cell, DNA methylation profile was not significantly different between the two IDH mutants (Figueroa et al., 2010a). Giving more support for IDH mutants having a causative role in increasing DNA methylation, engineered overexpression of mutant IDH1 or IDH2 both leads to increased 5-methylcytosine levels (Figueroa et al., 2010a). A mechanism for how the DNA hypermethylation phenotype arises in mutant IDH-expressing cells has been proposed and will be reviewed in section 6.2.

6. The role of 2-hydroxyglutarate in human malignancies

6.1 Genetic disorders that result in 2-hydroxyglutarate accumulation

With the identification of 2-HG as a novel tumor-associated metabolite, or oncometabolite, its effect on tumor cells has received increasing scrutiny. Two genetic defects have been described that involves accumulation of 2-HG and have been termed L-2- and D-2-hydroxyglutaric aciduria (L-2-HGA and D-2-HGA) based on which enantiomer of 2-HG accumulates (for reviews, see (Struys, 2006; Van Schaftingen et al., 2009)). They are rare neurometabolic disorders characterized by elevated 2-HG in bodily fluids including urine, plasma, and cerebrospinal fluid. L-2-HGA is the more common of the two. It is associated with L-2-HG dehydrogenase loss in virtually all cases resulting in the inability to oxidize L-2-HG to α-KG leading to L-2-HG accumulation. D-2-HGA is associated with either D-2-HG dehydrogenase loss or an R140 mutation in IDH2 both of which leads to D-2-HG accumulation (Kranendijk et al., 2010). L-2-HGA is the more severe of the two disorders and mainly affects the central nervous system with symptoms of hypotonia, tremors, and epilepsy that may progress to spongiform leukencephalopathy, muscular choreodystonia, mental retardation and psychomotor regression. Symptoms associated with D-2-HGA may be mild to nearly absent and include developmental delay, epilepsy, hypotonia, cardiomyopathy, and dysmorphic features. Interestingly, while IDH mutations result in D-2-HG accumulation, patients with L-2-HGA, and not D-2-HGA, have been reported to have a higher risk of developing malignant brain tumors (Aghili et al., 2009; Haliloglu et al., 2008). Thus mutant IDH likely has other effects beyond just production of the oncometabolite D-2-HG.

6.2 Inhibition of hydroxylases by 2-hydroxyglutarate

A major breakthrough in elucidating the function of 2-HG came with the discovery that this metabolite can inhibit the function of the TET dioxygenases (Xu et al., 2011). Members of the TET family have been shown to catalyze the conversion of 5-methylcytosine to 5-hydroxymethylcytosine (5-OH-MeC) in a reaction requiring α-KG, iron and oxygen (Ito et al., 2010; Tahiliani et al., 2009). While the physiologic significance of 5-OH-MeC has not been fully defined, it is believed to be an intermediate in the pathway that demethylates 5-methylcytosine. Thus, TET activity will result in decreased DNA methylation with potential widespread changes in gene expression. The first clue that 2-HG may be interacting with TET was the discovery that TET2 loss-of-function mutations seen in AML was mutually
exclusive with IDH1/2 mutations (Of 375 cases, 57 were IDH mutants, 28 were TET2 mutants, 0 were both IDH and TET2 mutants) (Figueroa et al., 2010a). This result suggests that IDH1/2 mutations and TET2 mutations have overlapping roles in AML pathogenesis. Forced expression of TET2 also resulted in increased 5-OH-MeC levels and this could be blocked by cotransfection with mutant but not wild type IDH1. Xu et al. has now shown that 2-HG can act as a competitive inhibitor of multiple α-KG-dependent dioxygenases including the TET family of 5-methylcytosine hydroxylases, histone demethylases and even prolyl hydroxylases (see section 5.2) (Xu et al., 2011). Using an in vitro enzymatic assay, D-2-HG could inhibit the activity of TET1 and TET2 reducing 5-OH-MeC in a dose-dependent fashion. Interestingly, L-2-HG, which is not produced by mutant IDH1, was actually even more effective at inhibiting the TET enzymes than the (D) enantiomer (Xu et al., 2011). This result may partially explain why malignant brain tumors were associated with the L-2-HGA but not the D-2-HGA genetic disorder.

6.3 Metabolic consequence of 2-hydroxyglutarate accumulation

As reviewed in section 5.1, direct treatment of cells with D-2-HG results in a metabolite profile, or metabolome that was similar to that found with mutant IDH1/2-expressing cells (Reitman et al., 2011). Of the 204 assessed biochemicals, from 107 to 130 were altered either up or down in the R132H-expressing or 2-HG-treated cells. Of these, 64 biochemical changes were shared between these cells. This number was much greater than the biochemical changes seen when comparing the R132H-expressing and the IDH1 knockdown cells where only 28 biochemicals were altered similarly. Likewise, mutant IDH2 (R172K) gave a profile similar to the R132H mutant. While these results suggest that D-2-HG is mediating some of the downstream effects of mutant IDH1/2, significant differences still exist thus highlighting 2-HG-independent effects of the mutant IDHs. One potential difference between these cells is that mutant IDH1 expression leads to glutamate depletion due to its conversion to α-KG and 2-HG while simply treating cells with 2-HG will not deplete glutamate. Some of the observed differences is consistent with this explanation as decreases in glutamate and several metabolites that are directly or indirectly derived from glutamate including glutathiones, N-acetylglutamate, N-acetyl-aspartyl-glutamate, α-KG, malate and fumarate are seen uniquely in mutant IDH1-expressing cells (Reitman et al., 2011). However, this explanation does not account for all the differences seen and mutant IDH expression is still likely to be causing some changes in the metabolite profile that is 2-HG-independent. These exact changes remain to be defined. The overall implication of these metabolite shifts on gliomagenesis is currently still largely unknown. However, there is an increasing recognition that changes in metabolism can have effects on tumorigenesis (for review, see (Vander Heiden et al., 2009)) and further research will likely begin to unravel the answers to these questions in the near future.

7. Diagnostic and therapeutic considerations with isocitrate dehydrogenase status in gliomas

7.1 Should routine testing for isocitrate dehydrogenase be performed?

Currently, determining the mutation status of IDH1/2 is not part of the standard molecular pathologic workup of gliomas. However, this is likely to change in the near future given the significant prognostic information the mutation status provides. With the high incidence of
The Role of Isocitrate Dehydrogenase Mutations in Glioma Brain Tumors

these IDH1/2 mutations in grade II and III gliomas (range from 66-83%) including astrocytomas, oligodendrogliomas and mixed oligoastrocytomas (Table 1), we would propose that all such tumors be assessed for these mutations. The incidence of these mutations is lower with GBMs so the decision to assess for such mutations remains an open question. Clearly, the incidence of IDH mutations in secondary GBMs is quite high, similar to that seen in lower grade gliomas. However, primary GBMs probably only contain IDH mutations in ~6% of cases (Table 1). Given this yield, we favor routine testing for IDH mutations in secondary but not primary GBMs. This view is based on the low potential yield of positive results in this GBM patient population and the fact that knowing the mutation status, while prognostic, will not alter the current therapeutic approach for these patients. However, if therapies that exploit the presence of these mutations are developed in the future, more comprehensive testing among all GBMs would be warranted.

7.2 Diagnostic tests used to detect mutant isocitrate dehydrogenase status
Determining the mutation status of IDH can be accomplished in a number of different ways. One standard approach is to detect for the mutant IDH protein. Currently, antibodies that recognize the mutant-specific epitope of the R132H IDH1 mutant have been reported (Capper et al., 2009; Kato et al., 2009). These antibodies have utility for western blotting of tumor cell lysates or immunohistochemical (IHC) staining of tumor tissue. While these methods utilize standard techniques familiar to pathology and molecular biology laboratories, they are limited by the fact that only the R132H mutant, which represents ~85% of IDH mutations in gliomas, can be identified (Table 2). Recently, an antibody specific for the altered epitope in the R132S IDH1 mutant was generated and found to be useful for western blotting and IHC staining (Kaneko et al., 2010). As more mutant-specific IDH1/2 antibodies become available, these antibodies and western blotting/IHC staining may be used to identify almost all potential cases with IDH1/2 mutations.

Alternatively, direct sequencing of the relevant regions in IDH1/2, or even the entire IDH1/2 genes, can be accomplished either from RNA or DNA harvested from the tumor. Improved genomic technologies have made these approaches very accessible both technically and costwise. Direct sequencing should theoretically provide 100% yield of mutations at the R132 residue of IDH1 and R140/R172 residues of IDH2. However, in practice, this may not be the case. Takano et al. reported their experience in comparing positive staining for IMab-1, an R132H-specific antibody, with results from routine direct sequencing of tumor DNA (Takano et al., 2010). In their study, only 9 of 12 cases detected as positive for expressing the R132H mutant on IHC staining turned out to be positive on initial screening with direct DNA sequencing. However, when the initial PCR product was subcloned, the IDH1-R132H could finally be detected in the three negative cases. This result indicates that tumor DNA was likely contaminated with a large amount of normal brain DNA making it difficult to detect the mutation on their initial screen. They conclude that IHC staining with the R132H antibody may be more sensitive than routine DNA sequencing for the detection of the IDH1-R132H mutant.

Finally, a mass spectrometry approach may be taken to detect for elevated D-2-HG in tumor lysate. This approach should permit detection of nearly all cases of mutant IDH1/2 since the neomorphic enzyme activity producing D-2-HG results with every mutation detected on R132 of IDH1 and R140/R172 of IDH2. Techniques for the detection both enantiomers of
2HG from urine or plasma using gas chromatography/mass spectrometry (GC/MS) or liquid chromatography/mass spectrometry (LC/MS) have been published and are routinely used by molecular genetics labs for the diagnosis of 2-hydroxyglutaric aciduria (Nyhan et al., 1995; Rashed et al., 2000). GC/MS and LC/MS have also been used by multiple groups in the detection of 2-HG from media and lysates of cells grown in culture as well as of tumor tissue (Dang et al., 2010; Ward et al., 2010; Xu et al., 2011). These techniques should be rapidly adaptable for clinical use in the detection of the 2-HG metabolite in tumor lysates.

7.3 Future directions for mutant isocitrate dehydrogenase diagnostic testing

Because 2-HG is produced in the media of cells expressing mutant IDH1/2, this metabolite may be a good biomarker for detection of disease, response to therapy, and, potentially, long-term followup assessing for disease control. Although there are no current reports of this approach, mass spectrometry analysis of cerebrospinal fluid (CSF) may potentially be utilized for detecting 2-HG produced by gliomas of varying grades. Examining CSF for 2-HG have been proposed as a reliable screen for the detection of this tumor-specific metabolite and plans are in place to investigate this in more detail (Van Meir, 2011). Similarly, 2-HG elevations may be detected by mass spectrometry in other bodily fluids such as serum or urine as alternative means of detecting for tumors that express mutant IDH1/2. Finally, others have proposed using magnetic resonance spectroscopic imaging (MRSI) as a potential approach for noninvasive detection of 2-HG within tumors seen on MRI (Mao, 2011). Again, while interesting, significant research on the use of MRSI for detection of 2-HG will need to be accomplished before clinical use can be contemplated.

7.4 Therapeutic targeting of mutant isocitrate dehydrogenase status

The specific IDH mutations is a very attractive target for therapeutic manipulations. A variety of therapeutics approaches can be realistically taken to target this mutation. One obvious approach would be to directly target the mutant enzyme. This can potentially be accomplished by utilizing approaches that alter the expression of the mutant enzyme or developing therapeutic inhibitors of its neomorphic enzyme activity. Alternatively, approaches that target some of the downstream effects of this enzyme activity can be taken. This might include counteracting the pseudohypoxic state that results from 2-HG inhibition of prolyl hydroxylases that induces HIF-1α, reversing partially or fully the hypermethylation phenotype that results from 2-HG inhibition of the 5-methylcytosine hydroxylases, etc. However, caution must be taken with approaches that target these changes since such patients have been shown to have a better prognosis than their counterparts without IDH mutations (Nobusawa et al., 2009; Sanson et al., 2009; Yan et al., 2009). The main concern is that cellular changes that result from IDH mutations is actually driving increased sensitivity to current therapies and reversing some effects of these mutations may decrease response to such therapies potentially worsening patient outcomes. In addition, since mutant IDH1/2 is thought to mainly have a role in tumor initiation and not necessarily in tumor maintenance, blocking mutant IDH1/2 activity or its downstream consequences may not be a particularly effective therapy. Therefore, any potential therapeutic regimen that targets mutant IDH1/2 and/or its downstream effects needs to be carefully evaluated to assure that such therapies are not detrimentally affecting outcomes.
7.5 Therapeutic targeting of a metabolic dependency seen in tumors that express mutant isocitrate dehydrogenase

An alternative therapeutic approach might be to target a “weakness” that results from the adaptation of the cell to the presence of IDH mutations. Seltzer et al. recently proposed an interesting idea along this line whereby they sought to target a specific metabolic dependency found in mutant IDH1-expressing tumors (Seltzer et al., 2010). Previous work had demonstrated that the predominant source of cytoplasmic α-KG for mutant IDH1 is glutamine (Dang et al., 2010), which is converted to glutamate by glutaminase and then to α-KG by either glutamate oxaloacetate transaminase or glutamate dehydrogenase. They reasoned that inhibition of glutaminase (GLS) could severely limit the availability of α-KG for production of 2-HG by mutant IDH1, which could potentially have anti-tumor effects in mutant IDH1-expressing cells. Exactly as predicted, inhibition of GLS either genetically with siRNA targeting GLS or chemically with bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide (BPTES) resulted in growth inhibition of mutant but not wild type IDH1-expressing cells (Seltzer et al., 2010). However, this explanation does not fully resolve all their data since they also found that inhibition of glutamine uptake by glutamine deprivation did not have a similar selective effect on mutant IDH1-expressing cells. They speculate that the growth inhibitory effect mediated by glutaminase inhibition may be dictated by alteration in the balance of specific metabolites that are not altered when glutamine is simply limited. Overall, this novel approach provides an example of a therapy that can be developed to specifically target the growth of mutant IDH1-expressing tumors by exploiting a metabolic dependency found in these tumors.

8. Conclusion

Even though IDH mutations were discovered less than 3 years ago, researchers have already gained a wealth of knowledge about how they are functioning. It is clear that IDH mutants lose their normal enzymatic activity and gain a new one that results in production of 2-HG. As we reviewed in this chapter, 2-HG appears to inhibit a number of α-KG-dependent hydroxylases which can have broad-ranging effects on a host of cellular functions. These include epigenetic changes with implications for having global effects on gene expression and changes in the stability of factors such as HIF-1α which can also change gene expression for hypoxia-related genes producing a pseudohypoxic state that may detrimentally affect treatment response. Researchers are probably just scratching the surface about the potential cellular effects of 2-HG. It is also apparent that mutant IDHs probably have cellular effects beyond just producing 2-HG. Many of these 2-HG-independent effects still remain to be determined. Currently, assessing for IDH1/2 mutations can be readily done in a number of ways but still only provides prognostic information. As we gain a greater understanding of the many cellular effects of mutant IDHs and start to link them with mechanisms involved in the initiation and maintenance of glial neoplasms, then more novel therapeutic approaches that exploit the presence of these mutations may be developed for these tumors.

9. References


The Role of Isocitrate Dehydrogenase Mutations in Glioma Brain Tumors


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Molecular Targets of CNS Tumors is a selected review of Central Nervous System (CNS) tumors with particular emphasis on signaling pathway of the most common CNS tumor types. To develop drugs which specifically attack the cancer cells requires an understanding of the distinct characteristics of those cells. Additional detailed information is provided on selected signal pathways in CNS tumors.

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