A pilot study correlating IDH-1/2 gene status with 2-Hydroxyglutarate concentration in plasma and urine from patients with glioma

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Disclosure

• No conflict of interest to declare
• Isocitrate dehydrogenase (IDH) 1 and 2 are enzymes involved in the citric acid cycle and catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate

• Mutations of IDH1 and IDH2 genes are found in 70-80% of grade II and III astrocytomas, oligodendrogliomas, oligoastrocytomas and secondary glioblastomas

• Mutations of IDH1 and IDH2 genes are considered to be independent prognostic factors in these patients

Gupta et al, JCP 2011

1 Dang et al, Nature 2009
2 Yan et al, NEJM 2009
• Mutations are typically found in **codon 132 in exon 4 of IDH1** and **172 in exon 4 of IDH2**. Of the mutations in IDH1, 95% are of a single variant R132H, resulting in substitution of arginine by histidine\(^1\)

• Mutations of IDH1 and IDH2 are mutually exclusive in glioma patients\(^1\)

\(^1\)Ichimura, Brain Tum Pathol 2012  
www.esmo2012.org
• Mutant IDH1 and IDH2 enzymes show a neomorphic enzymatic capacity to convert α-KG into D-2HG, which can be considered an oncometabolite\(^1\)

\(^1\)Prensner et al, Nature Medicine, 2011
Background

- Patients with IDH mutations have highly elevated amounts of intracellular D-2HG (up to 100-fold higher than wild-type IDH)\(^1\)

- **Tumor grade** does not correlate with intracellular 2HG concentration in IDH mutant gliomas \(^2\)

\(^1\)Jin et al, Plos One 2011
\(^2\)Dang Pope et al, J Neurooncol 2011
• In addition to intracellular 2HG accumulation, levels of other citric acid cycle intermediates such as glutamate, succinate and citrate can be altered in mutant IDH1/2-expressing cells\(^1\)

\(^1\)Reitman et al, PNAS 2011
Aims of the Study

• Investigate the effect of mutant and wild-type IDH1/2 genes on plasma and urinary 2HG concentration in patients with glioma

• Investigate the correlation between tumor volume/grade and 2HG concentration in plasma and urine

• Investigate whether mutant IDH1/2 may influence the plasma and urinary concentrations of other metabolites involved in the citric acid cycle
Methods

Major Inclusion Criteria:

- A prior biopsy/ surgery of the brain tumor and histological confirmation of glioma
- Neoplastic tissue available for analysis of IDH1/2 genes by PCR and sequence analysis
- A recent brain MRI (within 2 weeks) showing the neoplastic lesion
- Written consent

Major Exclusion Criteria:

- Absence of neoplastic lesions on brain MRI
- Any chemotherapy performed within 28 days prior
- Other neoplastic and metabolic diseases
- Renal and/or liver failure
Methods

• **Plasma and overnight-urine samples** were taken from all the patients

• **Exon 4 of IDH1/2 genes** were analyzed by Sanger sequencing

• **Metabolite concentrations** in urine and plasma were determined by liquid chromatography tandem mass spectrometry (LC-MS/MS)

• **Mann-Whitney test** was used to test for differences in metabolite concentrations between mutant and wild-type IDH1/2 patients

• **Tumor volume** was estimated based on Flair imaging for LGGs and on contrast-enhanced tumor area for HGGs using the formula for an ellipsoid
## Results

<table>
<thead>
<tr>
<th>Patients with mutant IDH1/2</th>
<th>Patients with wild type IDH1/2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td><strong>Sex</strong></td>
</tr>
<tr>
<td>5 Females, 8 Males</td>
<td>5 Females, 8 Males</td>
</tr>
<tr>
<td><strong>Average Age</strong></td>
<td><strong>Average Age</strong></td>
</tr>
<tr>
<td>51.5 ys</td>
<td>60 ys</td>
</tr>
<tr>
<td><strong>Histology</strong></td>
<td><strong>Histology</strong></td>
</tr>
<tr>
<td>11 HGGs (4 AO, 3 AOA, 1 AA, 3 GBM)</td>
<td>13 HGGs (1 AO, 1 AA, 11 GBM)</td>
</tr>
<tr>
<td>2 LGGs (grade 2 A, grade 2 OA)</td>
<td></td>
</tr>
<tr>
<td><strong>Mutations</strong></td>
<td><strong>Mutations</strong></td>
</tr>
<tr>
<td>IDH1_R132H (all patients)</td>
<td>none</td>
</tr>
<tr>
<td><strong>Average size of the tumor</strong></td>
<td><strong>Average size of the tumor</strong></td>
</tr>
<tr>
<td>64 cm³</td>
<td>61 cm³</td>
</tr>
</tbody>
</table>

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2HG concentration in plasma

No statistically significant difference of 2HG concentration between patients with mutant and wild-type IDH was detected \((p=0.25, \text{ Mann-Whitney test})\)
A statistically significant difference of urinary 2HG concentration between patients with mutant and wild-type IDH was detected \((p=0.03, \text{Mann-Whitney test})\).
A statistically significant difference in the ratio of 2HG concentrations in plasma and urine between patients with mutant and wild-type IDH was detected \((p=0.005, \text{Mann-Whitney})\)

\[
\begin{align*}
\text{mutated} & \quad 23.83 \pm 8.9 \\
\text{wild type} & \quad 13.96 \pm 7.6
\end{align*}
\]

*normalized by creatinine concentration*
No statistically significant correlation of plasma 2HG levels and tumor volume was detected \((p=0.56, \text{Spearman} \ r)\).
Although there is a trend for an association ($p=0.08$, Spearman $r$), no statistically significant correlation of urinary 2HG levels and tumor volume was detected.
Plasma 2HG concentration and tumor grade

![Plasma 2HG concentration and tumor grade graph](image)

p=0.09

Urinary 2HG concentration and tumor grade

![Urinary 2HG concentration and tumor grade graph](image)

p=0.71

No statistically significant differences of plasma and urinary 2HG concentrations among gliomas with different tumor grades.
Statistically significant differences of succinate, glutamate and citrate concentrations between patients with mutant and wild-type IDH were detected (Mann-Whitney test).
No statistically significant differences of succinate, glutamate and citrate concentrations between patients with mutant and wild-type IDH were detected (*Mann-Whitney test*)

*normalized by creatinine concentration

Concentrations of other metabolites in urine*
No significant correlations of plasma and urinary levels of the metabolites and tumor volume were detected (Spearman $r$)
Summary

• **Urinary 2HG concentration** in patients with mutant IDH was statistically lower than patients with wild-type IDH and there was a trend for a correlation with tumor size.

• No statistical difference in **plasma 2HG concentration** was observed between mutant and wild-type IDH and no association with tumor volume was found.

• In patients with mutant IDH **succinate, glutamate and citrate** had statistically higher plasma concentrations, but no associations with tumor volume were detected.

• **Tumor grade** did not correlate with plasma and urinary 2HG concentrations in mutant IDH gliomas.
Conclusions

This is the first study analyzing the concentrations of the metabolites involved in the citric acid cycle in both plasma and urine from glioma patients with mutant IDH gene.

However, a larger study is ongoing to draw final conclusions, in particular to analyze whether:

- These specific metabolic alterations might serve as surrogate markers for IDH mutations

- Urinary 2HG concentration might be used to monitor tumor growth and response to the treatment
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