Introduction

ImaBiotech investigated the metabolism of IDH-mutant gliomas by MALDI Mass Spectrometry Imaging (MALDI-MSI) to characterize the model and identify potential therapeutic targets. This work was performed on collected PDX (Patient-derived xenografts) with IDH wild type (IDHwt) and mutant (IDHm) tumors implemented in mouse cranium and grown until first appearance of neurologic signals before collection of the brains and sectioning.

The investigation on types and subtypes of tumors was driven by a targeted approach for following key molecular features in oncology but also a differential approach via statistical analysis to reveal unique features for each tumor and their impact on different biological pathways including the energetic balance, the oxidation stress pathway but also the lipid composition and the neurotransmitter alterations.

Solution

Gliomas represent 40% of the primary brain tumors and have bad prognosis. Mutation of the isocitrate dehydrogenase (IDH) is found to occur in 70-80% of the lower grade gliomas. This mutation is associated with a better survival rate compared to the wild type version. From a molecular point of view, the wild type version of IDH (IDHwt) converts the isocitrate into α-Ketoglutarate (α-KG) using the NADP+ enzymatic complex, α-KG being a key player of the TCA cycle for ATP production. When the enzyme harbors a mutation (IDHm), it now overexpresses the oncometabolite 2-Hydroxyglutarate (2-HG) from α-KG, using this time NADPH, leading to the hypermethylation of the DNA and the chromatin (Figure 1).

However, the underlying mechanisms in the metabolic or lipid landscape of these tumors remain unknown to identify vulnerabilities and consequently to identify therapeutic targets.

Luxembourg Institute of Health was able to grow such PDX including the major mutated IDH1 (IDH1m) form and the wild type IDH1 (IDH1wt) in oligodendroglioma and glioblastoma multiforme (GBM) type for investigation by MALDI Mass Spectrometry Imaging (MSI) to highlight the molecular features and differences for both models as well as potential prognosis biomarkers.

Results

In the three IDH1m subtypes, relevance of the oncology marker 2-HG was demonstrated by an intense detection of the molecule in the tumor regions of the brains almost confined exclusively to these regions along with a certain heterogeneity (Figure 2). Complementary analyses using an electrospray source revealed a 50 fold intensity ratio between wild type and mutant tumor regions showing thus the overexpression of this metabolite in the mutant models.
ImaBiotech investigated the metabolism of IDH-mutant gliomas by MALDI Mass Spectrometry Imaging (MALDI-MSI) to characterize the model and identify potential therapeutic targets. This work was performed on collected PDX (Patient-derived xenografts) with IDH wild type (IDHwt) and mutant (IDHm) tumors that were implemented in mouse cranium and grown until first appearance of neurologic signals before collection of the brains and sectioning.

This investigation on types and subtypes of tumors was driven by a targeted approach for following key molecular features in oncology but also a differential approach via statistical analysis to reveal unique features for each tumor and their impact on different biological pathways including the energetic balance, the oxidation stress pathway but also the lipid composition and the neurotransmitter alterations.

<table>
<thead>
<tr>
<th>Model</th>
<th>Patient-derived xenografts (PDX)</th>
<th>Type</th>
<th>H&amp;E Staining</th>
<th>2-HG Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDH1m</td>
<td>E478</td>
<td>Oligodendroglioma grade III with 1p/19q co-deletion</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>E478</td>
<td>Anaplastic oligodendroglioma grade III no 1p/19q co-deletion</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T394</td>
<td>GBM Grade IV with partial 1p/19q co-deletion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDH1wt</td>
<td>P3</td>
<td>GBM Grade IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P8</td>
<td>GBM Grade IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T434</td>
<td>GBM Grade IV</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Energetic levels were characterized by the high glucose uptake in tumor regions of IDHm and IDHwt and by adenosine phosphate levels including the ATP, ADP and AMP. The latter was the only one showing an equal levels compared to the control brain. ATP and AMP were increased in both tumors and even higher for the wild type model. Resulting energy charge index calculation based on these metabolites showed a lower value for the mutant model than wild type one in accordance with the limitation of the proliferation of tumor cells in the mutant model and thus better associated survival rate (Figure 3). Despite opposite mechanisms to recycle the NADPH/NADP+ enzymatic complex (Figure 1), both types of tumors showed similar increased GSH levels characterizing a similar oxidative stress and thus a certain adaptation of IDHm. Deeper investigation highlighted some important changes of metabolites involved in the transsulfuration pathway producing the cysteine, the precursor of GSH synthesis. This included the cystathionine, overexpressed in the tumors and lower in the mutant model (Figure 4). Further investigation of CBS enzyme involved in the synthesis of Cystathionine was showed upregulated in IDHm and higher for a specific mutant subtype including a codeletion of the genes 1p/19q (E478). The enzyme was demonstrated as novel prognostic biomarker of this subtype.
Important amino acid and neurotransmitter changes were also found by the untargeted approach with the extinction of the aspartate signal in the tumor regions. Synthesis intermediate specie NAA for the synthesis of NAAG was also found extinguished in both tumors compared to the control brain (Figure 5). More importantly, the neurotransmitter NAAG was overexpressed only in the mutant version owing the co-deleted genes 1p/19q and thus could potentially be used a novel prognostic biomarker for this subtype. Finally, aberrant lipid composition was pointed out by the untargeted approach revealing for example grade-dependent lipid expressions such as m/z 778.510, potentially identified as PS(37:4) lipid, present in the two grade III IDHm and absent from grade IV IDHm (Figure 6).

MALDI Mass Spectrometry Imaging correlated the expression of the IDH1 mutant or wild type versions through major molecular pathways with the histology of the tumor regions. The technique was evenable to identify major differences in IDHm subtypes to highlight potential prognosis markers, particularly for the version with co-deleted genes 1p/19q with high CBS expression or overexpressed neurotransmitter NAAG. The imaging data also discriminated subregions in the tumors due to certain heterogeneity of molecular signals meaning that further investigation at higher spatial resolution would bring more details to the interactions between tumor cells and their environment.

**Authors**
- Guillaume Hochart
- Fred Fack (Luxembourg Institute of Health)
- Saverio Tardito (Beatson Institute)
- Edma Fontaine
- Simone Niclou (Luxembourg Institute of Health)
- Jonathan Stauber