Gain of 12p encompassing CCND2 is associated with gemistocytic histology in IDH mutant astrocytomas

Felix Sahm1,2, Andrey Korshunov3, Daniel Schrimpf1,2, Damian Stichel1,3, David T.W. Jones3, David Capper1,2, Christian Koelsche1,2, David Reuss1,2, Annekathrin Kratz1,2, Kristin Huang1,2, Annika K. Wefers1,2, Matthias Schick4, Melanie Bewerunge-Hudler5, Michel Mittelbronn5, Michael Platten6,7,8, Daniel Hänggi9, Astrid Jeibmann10, Andreas Unterberg11, Christel Herold-Mende11, Stefan M. Pfister1,12, Sebastian Brandner13, Wolfgang Wick6,14, Andreas von Deimling1,2

1. Department of Neuropathology, Institute of Pathology, Ruprecht-Karls-University Heidelberg, Heidelberg, Germany
2. Clinical Cooperation Unit Neuropathology, German Consortium for Translational Cancer Research (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany
3. Division of Pediatric Neurooncology, German Consortium for Translational Cancer Research (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany
4. Genomics and Proteomics Core Facility, Microarray Unit, German Cancer Research Center (DKFZ), Heidelberg, Germany
5. Department of Neuropathology (Edinger Institute), University Hospital Frankfurt, Frankfurt, Germany
6. Neurology Clinic, University Hospital Heidelberg, Heidelberg, Germany
7. Clinical Cooperation Unit Neuroimmunology and Brain Tumor Immunology, German Consortium for Translational Cancer Research (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany
8. Neurology Clinic, University Hospital Mannheim, Mannheim, Germany
9. Department of Neurosurgery, University Hospital Mannheim, Mannheim, Germany
10. Institute of Neuropathology, University Hospital Münster, Münster, Germany
11. Department of Neurosurgery, University Hospital Heidelberg, Heidelberg, Germany
12. Department of Pediatric Oncology, Haematology and Immunology, Heidelberg University Hospital, and National Center for Tumor Diseases (NCT), Heidelberg, Germany
13. Division of Neuropathology of the UCL Institute of Neurology in London, London, UK
14. Clinical Cooperation Unit Neurooncology, German Consortium for Translational Cancer Research (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany

Gemistocytic astrocytoma represents a small subgroup accounting for approximately 5% of diffuse astrocytic glioma. The WHO classification defines gemistocytic astrocytoma by presence of “a conspicuous, though variable, fraction of gemistocytic neoplastic astrocytes” [5]. These cells should account for at least 20% of the tumor cells. The term “gemistocytic” was coined by Nissl in 1904 for cells with homogeneous, faintly eosinophilic cytoplasms with short branching processes. It originates from the German word “gemästet” (filled, stuffed, swollen), sometimes also referred back to the Greek “gemistos” with similar meaning.

The neoplastic nature of gemistocytic cells in astrocytoma could be clearly demonstrated by binding of an IDH1R132H mutant protein specific antibody [1]. However, so far no molecular drivers characteristic for this gemistocytic differentiation have been identified. Previous molecular analyses reported higher frequencies of TP53 and PTEN mutations [14], lower frequency of IDH mutations, and alterations in RAS5 and ERCC1 [7]. Several studies found shorter progression-free-survival in gemistocytic astrocytoma cases compared to fibrillary astrocytoma [4,8-10], whilst others did not confirm this finding [a reference should be given here]. However, there appears to be a contradiction between the accelerated progression and lower proliferative activity of gemistocytes compared to other tumor cells in the same sample, or in general in diffuse glioma [2-4,15].

To identify potentially recurrent alterations associated with gemistocytic morphology in astrocytoma we performed high-throughput high-resolution genetic and epigenetic analysis on a set of 24 gemistocytic astrocytomas. The control group consisted of 47 IDH mutant astrocytomas WHO grade II, 104 IDH mutant anaplastic astrocytomas WHO grade III, and 293 IDH wild-type glioblastomas WHO grade IV.

The Illumina Infinium HumanMethylation450 BeadChip (450k) array data was used for methylation profiling and to calculate a low-resolution copy number profile (CNP) as previously described [12].
Targeted re-sequencing was performed on the genes and with the technology as reported previously [11].

A distinctive feature of tumours with gemistocytic histology was a recurrent numerical aberration in the telomeric region of chromosomal arm 12p, encompassing CCND2 (Figure 1A). An integrated analysis of copy-number variation (Stichel et al., in preparation) in all gemistocytic astrocytoma of our cohort also indicated 12p as the most consistently altered locus (Figure 1B). In particular, focal gain of CCND2 and adjacent regions was seen in 8 of 9 gemistocytic astrocytomas WHO grade II and in 13 of 15 anaplastic astrocytomas with distinct gemistocytic morphology (Table 1). Instead, this alteration was observed in only 5 of 47 fibrillary astrocytomas WHO grade II and in only 19 of 104 anaplastic astrocytomas lacking gemistocytic morphology. The alterations detected by analysis of copy number plots based on 450K analysis were confirmed by FISH in a subset of 18 cases (11 gemistocytic cases, 7 fibrillary cases), yielding concordance in 17 out of 18 (94%) cases (Figure 1B). The single non-concordant case was an anaplastic gemistocytic astrocytoma without indications of chromosome 12 gain by 450k but low-level gain detected by FISH probe directed against 12p12 encompassing CCND2.

This difference was highly significant within grade II and grade III gliomas, respectively (each p<0.0001, Fisher’s exact test). Also, the event of 12p/CCND2 gain was significantly associated with gemistocytic histology over the entire diffuse astrocytic glioma cohort (p<0.0001, Table 1). Unsupervised clustering of methylome profiles from gemistocytic and fibrillary astrocytoma did not separate these from each other (data not shown).

To assess the mutational landscape of gemistocytic astrocytoma, 17 tumours were further analysed by panel sequencing. All cases harboured IDH1R132 mutations (16/17 IDH1R132H, 1/17 IDH1R132G) and TP53 mutations. Other recurrently mutant genes were ATRX (10/17), ALK (2/17), CSF1R (2/17), FGFR1 (2/17), GSE1 (2/17), MSH6 (2/17), NF1 (2/17), and SMO (2/17, not affecting the activating hotspots). These findings are in line with studies describing high rates of TP53 mutations in gemistocytic astrocytomas, but contrasts reports on high frequencies of PTEN mutations of which none was found in the present set.

Recent reports suggested aberrations of copy number and methylation of ERCC1 and RRAS as a possible marker for gemistocytic astrocytoma [7]. However, we could not detect these aberrations in our dataset (mean beta values for ERCC1 promoter sites 0.09 and 0.1, for RRAS promoter sites 0.08 and 0.09, in gemistocytic and control samples, respectively).

Upregulation of CCND2 due to higher copy abundance also provides an explanation for several prior observations on this sub-entity: CCND2 is physiologically upregulated in radial glial cells of the subventricular zone during brain development, and activating CCND2 mutations result in megalencephaly whilst abrogation of CCND2 leads to microcephaly [6]. The higher abundance of CCND2 protein might also disrupt the regular cell cycle, preventing the transition from S to G2 phase, and explain lower mitotic activity but higher pleomorphism with higher number of multi-nucleated cells in such cases. Moreover, the recent approval of inhibitors of the CDK4/6 axis [13], both interacting with CCND2, also opens an additional therapeutic approach for this glioma subtype.
Table 1 Subtypes of diffuse glioma and 12p status

<table>
<thead>
<tr>
<th>Subtype</th>
<th>12p gain</th>
<th>12p balanced/del</th>
</tr>
</thead>
<tbody>
<tr>
<td>All AII (56)</td>
<td>13 (23%)</td>
<td>43 (77%)</td>
</tr>
<tr>
<td>All gem (9)</td>
<td>8 (89%)</td>
<td>1 (11%)</td>
</tr>
<tr>
<td>All non-gem (47)</td>
<td>5 (11%)</td>
<td>42 (89%)</td>
</tr>
<tr>
<td>All AIII (119)</td>
<td>29 (24%)</td>
<td>90 (76%)</td>
</tr>
<tr>
<td>All gem (15)</td>
<td>13 (87%)</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>All non-gem (104)</td>
<td>19 (18%)</td>
<td>85 (82%)</td>
</tr>
<tr>
<td>All GBM (293)</td>
<td>32 (11%)</td>
<td>261 (89%)</td>
</tr>
</tbody>
</table>

Figure 1

Figure 1 Representative copy-number profile of a gemistocytic astrocytoma (A). Integrated copy-number analysis across all gemistocytic astrocytoma (B). Fluorescence in-situ hybridization of a case with amplification (left) and low-level gain of CCND2 (middle, higher magnification right).
References


