**GOPC:ROS1 and other ROS1 fusions represent a rare but recurrent drug target in a variety of glioma types**

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Gliomas are the most common primary tumors of the central nervous system (CNS). Among low-grade gliomas, mitogen-activated protein kinase (MAPK) pathway alterations are frequent and may provide a therapeutic target. Currently, mechanism-of-action based therapeutic approaches outside the MAPK pathway are scarce. However, especially patients with subtotally resected, recurrent or highly malignant tumors may substantially benefit from the identification of...
cases or small series [3, 5, 8, 9]. Data on fusions in glioma are limited to single
involved in chromosomal rearrangements in various can-
insight into disease pathogenesis, but also offer targets for
additional specific oncogenic drivers that not only provide
fusions are not specific to any one glioma entity. Interest-
neuronal tumor, high-grade glioma, PF
other abbreviations: LGG/LGNT low-grade glioma/low-grade gli-
-ration class infantile hemispheric glioma, other tumors clus-
ng RNA sequencing in a diagnostic context are highlighted in blue.
fusion (with exons 1–2 (NM_0017612), ARCN1 exons 1–5 (NM_001655), or
exons 35–43 of ROS1 (NM_002944). Single cases of
exons 36–43 of ROSI fused downstream of ZCCHC8 exons
exons 35–43 of ROS1 (NM_0017812). In all fusion events, the kinase domain of
ARCN1:ROS1 fusion (with exons 1–12 of ARCN1 (NM_0017812), CHCHD3
were already detected as such by perform-
neural tumor, GBM glioblastoma, PF posterior fossa, N/A not available
ROS1:ROS1 fusion detected in case #3 involving exons 1–7 of GOPC and exons 35–43 of ROSI (b). Circos plot of
gene fusions targeting ROSI (lines link fusion gene partners accord-
ing to chromosomal location; c). t-distributed stochastic neighbor
ROS1-fused glioma alongside selected reference samples (d). Reference
DNA methylation classes: posterior fossa pilocytic astrocytoma (LGG, PA PF), hemispheric pilocytic astrocytoma and gangli-
neurotoma IDH wildtype subclass RTK II (GBM, RTK II), glioblas-
toma IDH wildtype subclass mesenchymal (GBM, MES). The two ROSI-
fused glioma samples that were already detected as such by perform-
ing RNA sequencing in a diagnostic context are highlighted in blue. Other abbreviations: LGG/LGNT low-grade glioma/low-grade gli-
neuronal tumor, HGG high-grade glioma, GBM glioblastoma, PF posterior fossa, N/A not available.

Recently, an enrichment of these fusions (about 7%) was found in a small number of mostly gliomas in infants [2, 7]. Routine diagnostic assessment of ROSI status in gliomas, however, is so far restricted to a few specialized centers or molecularly informed trials [11]. Thus, the landscape of ROSI fusions across a broad series of glial tumors of all age groups has not been comprehensively studied so far. Consequently, the distribution among the various types of low- to high-grade glioma is unknown. Similarly, no data exist to determine whether ROSI fusion-positive gliomas, irrespective of histology, may share further biological features, potentially supporting a ‘ROSI-subtype’ of gliomas. Here, we investigated the presence of ROSI fusions in a large cohort of 20,723 patients encompassing different diagnostic entities within the spectrum of glioma, to elucidate the frequency of such fusions and the characteristics of the respective cases.

To identify gliomas with structural alterations affecting chromosome 6q (around the ROSI locus), we systematically evaluated copy-number data of our DNA methylation dataset encompassing 20,723 gliomas, irrespective of specific entity and WHO grade (Supplementary Fig. 1 and 2, online resource). As a high proportion of ROSI fusions (in particular the most frequent GOPC:ROS1 fusion) are accompanied by a segmental loss of chromosome 6q22 in the copy-number profile, DNA methylation data were screened for a segmental loss covering that region (Supplementary Fig. 1, online resource). Automated analysis was followed by visual inspection and led to the identification of 14 potential cases. On suspicious cases, we performed RNA and targeted exome sequencing, and confirmed the presence of ROSI fusions in all 14 tumors (Fig. 1a and Supplementary Table 1). In the most common (n = 11) GOPC:ROS1 fusions (Fig. 1b), exons 1–7 or 1–4 of GOPC (NM_001017408) are fused in frame to exons 35–43 of ROSI (NM_002944). Single cases of exons 36–43 of ROSI fused downstream of ZCCHC8 exons 1–2 (NM_0017612), ARCN1 exons 1–5 (NM_001655), or CHCHD3 exons 1–2 (NM_017812) were also observed (Fig. 1c). In all fusion events, the kinase domain of ROSI was retained (Fig. 1b and Supplementary Table 2). ROSI transcript levels were upregulated in all ROSI-fused gliomas (Supplementary Fig. 3, online resource). Interestingly, ROSI partners are associated with very different cellular functions, including, e.g., intracellular protein trafficking and RNA processing and degradation. In addition, two further ROSI-fused glioma samples that were already detected as such by performing RNA sequencing in a diagnostic context, after the initial screen was performed were included into subsequent analyses. One of the samples harbored a GOPC:ROS1 fusion (with exons 1–7 of GOPC fused to exons 35–43 of ROSI) and indeed showed segmental loss of chromosome 6q22, while the other case harbored a CEP85L:ROS1 fusion (with exons 1–12 of CEP85L (NM_001042475) fused to exons 35–43 of ROSI) with a segmental gain of chromosome 6q22. In addition, we analyzed RNA sequencing data from a set of > 1000 FFPE tissue samples processed in a diagnostic setting. Here, no further gliomas harboring a ROSI-fusion were detected.

A t-distributed stochastic neighbor embedding (t-SNE) analysis of DNA methylation profiles alongside a broad reference set of CNS tumors [1] revealed that the ‘ROS1 cohort’ molecularly segregated into different glioma groups (Fig. 1d). Six of the samples grouped with the DNA methylation class infantile hemispheric glioma, other tumors clustered with various reference classes of glioma from low- to high-grade (Fig. 1d). Histological re-evaluation confirmed the different histological entities and underline that ROSI fusions are not specific to any one glioma entity. Interestingly, most of the patients harboring a fusion were children (particularly infants). Of note, however, was the finding that two classical adult IDH-wildtype glioblastomas in adult patients also harbored a GOPC:ROS1 fusion.
Our data show a high frequency of ROS1 gene fusions within the DNA methylation class infantile hemispheric glioma, which is in line with recent studies [2, 7]. This clinically distinct group of gliomas (that were initially often diagnosed as glioblastomas) carries a high prevalence of gene fusions with ROS1, ALK, NTRK1/2/3, or MET as a fusion partner. However, our finding that ROS1 fusions also occur in cases that were both histologically and epigenetically clearly pilocytic astrocytoma or IDH-wildtype glioblastoma, respectively, underscores that this event is not pathognomonic for infantile hemispheric glioma, nor limited to pediatric patients, so in that respect concerns a quite ‘promiscuous’ marker.

Although relatively rare in other gliomas, identification of ROS1 fusions is important from a treatment perspective, as there are specific inhibitors available. Screening via copy-number profiling and subsequent validation using RNA sequencing provides an efficient approach to identify patients who may benefit from this targeted therapy. However, as illustrated by one of the cases that was identified by performing RNA sequencing in a diagnostic setting, not all variants of ROS1 fusion necessarily show a deletion around the ROS1 locus. For example, copy-neutral translocations can lead to ROS1 fusions as well, and such cases would be missed by screening for segmental 6q22 loss. RNA sequencing thus remains the ‘gold standard’ for adequate detection of these rare events. However, it should be noted that tumor heterogeneity and blood–brain barrier permeability of specific ROS1-inhibitors could be one of the major problems limiting the efficacy of targeted therapies.

Our findings highlight ROS1 fusions as a rare but potentially highly relevant therapeutic target for a subset of patients with gliomas of different histological grades and biological classes. Even though these fusions have no strong diagnostic relevance, since they are not pathognomonic for a tumor type, they are in line with the increasing demand to provide predictive markers in diagnostic neuropathology. This highlights the need for expanded testing for such alterations beyond infant gliomas. It will be interesting to see whether ROS1-inhibitors will be effective in upcoming clinical trials for glioma patients.

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