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CORRESPONDENCE

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

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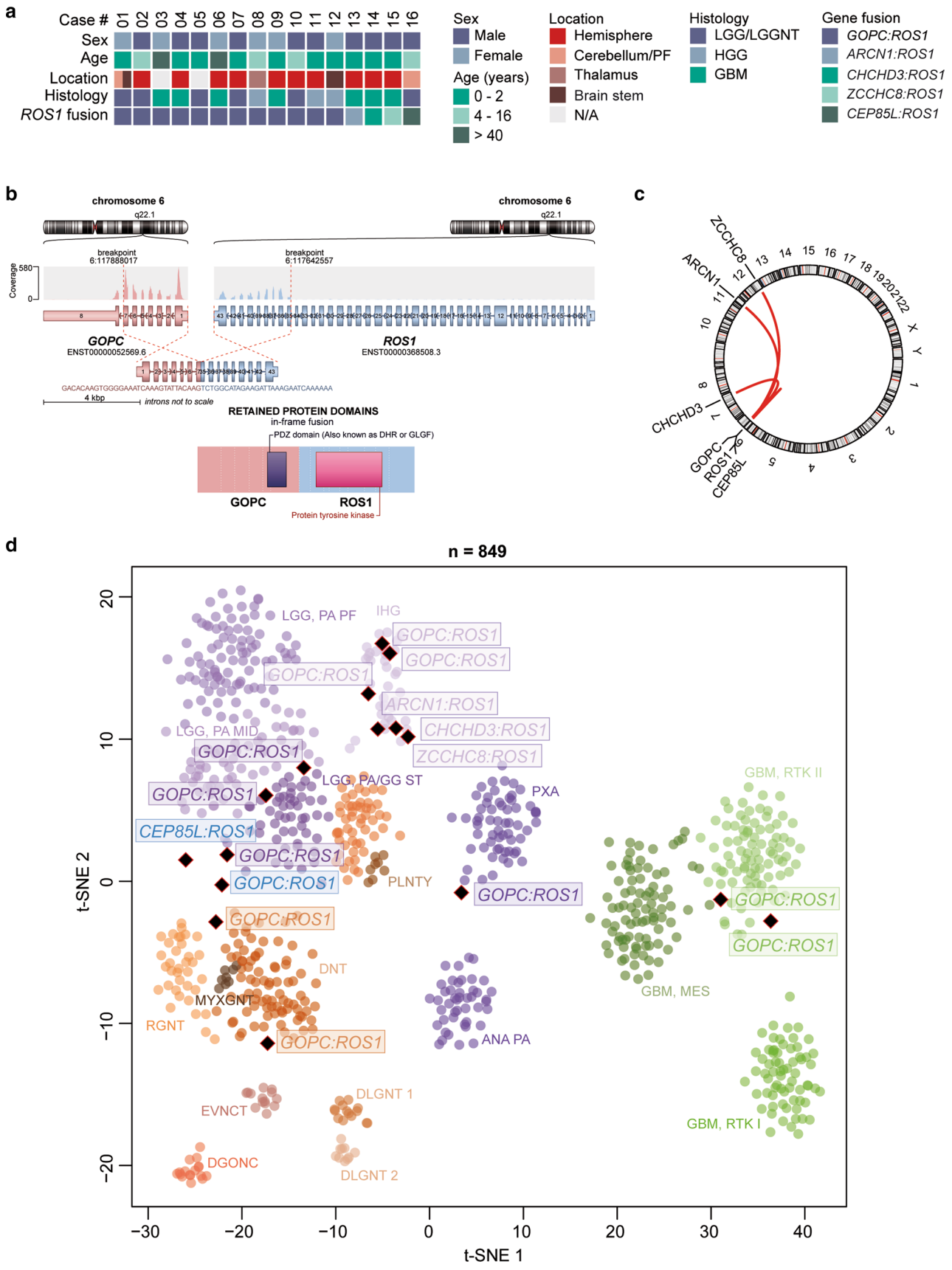
a
Case # 01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16
Sex
Age
Location
Histology
ROS1 fusion
Sex
Male
Female
Age (years)
0 - 2
4 - 16
> 40
Location
Hemisphere
Cerebellum/PF
Thalamus
Brain stem
N/A
Histology
LGG/LGGNT
HGG
GBM
Gene fusion
GOPC:ROS1
ARCN1:ROS1
CHCHD3:ROS1
ZCCHC8:ROS1
CEP85L:ROS1
b
chromosome 6
q22.1
breakpoint 6:117888017
breakpoint 6:117642557
Coverage
580
0
GOPC
ENST00000052569.6
ROS1
ENST00000368508.3
GACACAAGTGGGGAAATCAAAGTATTACAAGTCTGGCATAGAAGATTAAAGAATCAAAAAA
4 kbp
introns not to scale
RETAINED PROTEIN DOMAINS
in-frame fusion
PDZ domain (Also known as DHR or GLGF)
GOPC
ROS1
Protein tyrosine kinase
c
ZCCHC8
ARCN1
CHCHD3
GOPC
ROS1
CEP85L
d
n = 849
t-SNE 2
t-SNE 1
LGG, PA PF
IHG
LGG, PA MID
LGG, PA/GG ST
PXA
PLNTY
DNT
MYXGNT
RGNT
ANA PA
EVNCT
DLGNT 1
DLGNT 2
DGONC
GBM, RTK II
GBM, MES
GBM, RTK I
GOPC:ROS1
ARCN1:ROS1
CHCHD3:ROS1
ZCCHC8:ROS1
CEP85L:ROS1

◂**Fig. 1** Summary of clinico-pathological characteristics and key molecular findings in tumors with *ROS1* gene fusion (**a**). Schematic illustration of the *GOPC:ROS1* fusion detected in case #3 involving exons 1–7 of *GOPC* and exons 35–43 of *ROS1* (**b**). Circos plot of gene fusions targeting *ROS1* (lines link fusion gene partners according to chromosomal location; **c**). t-distributed stochastic neighbor embedding (t-SNE) analysis of DNA methylation profiles of *ROS1*-fused glioma alongside selected reference samples (**d**). Reference DNA methylation classes: posterior fossa pilocytic astrocytoma (LGG, PA PF), hemispheric pilocytic astrocytoma and ganglioglioma (LGG, PA/GG ST), midline pilocytic astrocytoma (LGG, PA MID), polymorphous low-grade neuroepithelial tumor of the young (PLNTY), diffuse leptomeningeal glioneuronal tumor subgroup 1 (DLGNT 1), diffuse leptomeningeal glioneuronal tumor subgroup 2 (DLGNT 2), infantile hemispheric glioma (IHG), extraventricular neurocytoma (EVNCT), dysembryoplastic neuroepithelial tumor (DNT), rosette-forming glioneuronal tumor (RGNT), myxoid glioneuronal tumor of the septum pellucidum and lateral ventricle (MYXGNT), diffuse glioneuronal tumor with oligodendroglioma-like features and nuclear clusters (DGONC), anaplastic astrocytoma with piloid features (ANA PA), pleomorphic xanthoastrocytoma (PXA), glioblastoma IDH wildtype subclass RTK I (GBM, RTK I), glioblastoma IDH wildtype subclass RTK II (GBM, RTK II), glioblastoma IDH wildtype subclass mesenchymal (GBM, MES). The two *ROS1*-fused glioma samples that were already detected as such by performing RNA sequencing in a diagnostic context are highlighted in blue. Other abbreviations: *LGG/LGGNT* low-grade glioma/low-grade glioneuronal tumor, *HGG* high-grade glioma, *GBM* glioblastoma, *PF* posterior fossa, *N/A* not available

additional specific oncogenic drivers that not only provide insight into disease pathogenesis, but also offer targets for personalized cancer therapies. The ROS proto-oncogene 1 (*ROS1*) gene encodes a receptor tyrosine kinase that is involved in chromosomal rearrangements in various cancers [6], which present an attractive therapeutic target, since specific inhibitors have been approved for several entities [4, 10]. Data on *ROS1* fusions in glioma are limited to single cases or small series [3, 5, 8, 9].

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 *ROS1* status in gliomas, however, is so far restricted to a few specialized centers or molecularly informed trials [11]. Thus, the landscape of *ROS1* 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 *ROS1* fusion-positive gliomas, irrespective of histology, may share further biological features, potentially supporting a 'ROS1-subtype' of gliomas. Here, we investigated the presence of *ROS1* 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 *ROS1* 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 *ROS1* 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 *ROS1* 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 *ROS1* (NM_002944). Single cases of exons 36–43 of *ROS1* 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 *ROS1* was retained (Fig. 1b and Supplementary Table 2). *ROS1* transcript levels were upregulated in all *ROS1*-fused gliomas (Supplementary Fig. 3, online resource). Interestingly, *ROS1* partners are associated with very different cellular functions, including, e.g., intracellular protein trafficking and RNA processing and degradation. In addition, two further *ROS1*-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 *ROS1*) 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 *ROS1*) 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 *ROS1*-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 *ROS1* 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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References

1. Capper D, Jones DTW, Sill M, Hovestadt V, Schrimpf D, Sturm D et al (2018) DNA methylation-based classification of central nervous system tumours. Nature 555:469–474. https://doi.org/10.1038/nature26000
2. Clarke M, Mackay A, Ismer B, Pickles JC, Tatevossian RG, Newman S et al (2020) Infant high grade gliomas comprise multiple subgroups characterized by novel targetable gene fusions and favorable outcomes. Cancer Discov. https://doi.org/10.1158/2159-8290.CD-19-1030
3. Cocce MC, Mardin BR, Bens S, Stutz AM, Lubieniecki F, Vater I et al (2016) Identification of ZCCHC8 as fusion partner of ROS1 in a case of congenital glioblastoma multiforme with a t(6;12)(q21;q24.3). Genes Chromosomes Cancer 55:677–687. https://doi.org/10.1002/gcc.22369
4. Das A, Cheng RR, Hilbert ML, Dixon-Moh YN, Decandio M, Vandergrift WA 3rd et al (2015) Synergistic effects of crizotinib and temozolomide in experimental FIG-ROS1 fusion-positive glioblastoma. Cancer Growth Metastasis 8:51–60. https://doi.org/10.4137/CGM.S32801
5. Davare MA, Henderson JJ, Agarwal A, Wagner JP, Iyer SR, Shah N et al (2018) Rare but recurrent ROS1 fusions resulting from chromosome 6q22 microdeletions are targetable oncogenes in glioma. Clin Cancer Res 24:6471–6482. https://doi.org/10.1158/1078-0432.CCR-18-1052
6. Davies KD, Doebele RC (2013) Molecular pathways: ROS1 fusion proteins in cancer. Clin Cancer Res 19:4040–4045. https://doi.org/10.1158/1078-0432.CCR-12-2851
7. Guerreiro Stucklin AS, Ryall S, Fukuoka K, Zapotocky M, Lassaletta A, Li C et al (2019) Alterations in ALK/ROS1/NTRK/MET drive a group of infantile hemispheric gliomas. Nat Commun 10:4343. https://doi.org/10.1038/s41467-019-12187-5
8. Nakano Y, Tomiyama A, Kohno T, Yoshida A, Yamasaki K, Ozawa T et al (2019) Identification of a novel KLC1-ROS1 fusion in a case of pediatric low-grade localized glioma. Brain Tumor Pathol 36:14–19. https://doi.org/10.1007/s10014-018-0330-3
9. Richardson TE, Tang K, Vasudevaraja V, Serrano J, William CM, Mirchia K et al (2019) GOPC-ROS1 fusion due to microdeletion at 6q22 is an oncogenic driver in a subset of pediatric gliomas and glioneuronal tumors. J Neuropathol Exp Neurol 78:1089–1099. https://doi.org/10.1093/jnen/nlz093
10. Shaw AT, Ou SH, Bang YJ, Camidge DR, Solomon BJ, Salgia R et al (2014) Crizotinib in ROS1-rearranged non-small-cell lung cancer. N Engl J Med 371:1963–1971. https://doi.org/10.1056/NEJMoa1406766

11. Wick W, Dettmer S, Berberich A, Kessler T, Karapanagiotou-Schenkel I, Wick A et al (2019) N2M2 (NOA-20) phase I/II trial of molecularly matched targeted therapies plus radiotherapy in patients with newly diagnosed non-MGMT hypermethylated glioblastoma. Neuro Oncol 21:95–105. https://doi.org/10.1093/neuonc/noy161