Posterior fossa pilocytic astrocytomas with oligodendroglial features show frequent *FGFR1* activation via fusion or mutation

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Low-grade glial (LGG) and glioneuronal tumors (LGGNT) of the central nervous system (CNS) are an extremely diverse group of tumors with overlapping morphological features [6], making their histological diagnosis sometimes challenging. Molecular analyses can be very useful in distinguishing these entities from their clinical or histological mimics and raises the possibility of personalized targeted therapy [7,15]. In recent years, genome-wide DNA methylation profiling has proven to be a powerful technique to distinguish biological subgroups of brain tumors with characteristic alterations [1,3,11].

We investigated a challenging diagnostic case of a low-grade neuroepithelial tumor (LGNT) with focally discrete rosettes located in the mesencephalon by DNA methylation profiling, and subsequently identified a small group of neoplasms (n=9) in a cohort of > 30,000 tumors that were distinct from established DNA methylation profiles of other entities. Analyses and tissue acquisition are described in the supplementary material. Unsupervised hierarchical clustering and t-distributed stochastic neighbor embedding (t-SNE) analysis of DNA methylation patterns alongside other, well-characterized LGNT entities, confirmed the distinct nature of this class (Fig.1). Analysis of copy number profiles (CNPs) showed evidence for a fusion between *fibroblast growth factor receptor 1 (FGFR1)* and *transforming acidic coiled-coil containing protein 1 (TACC1)* genes (Fig.1) in four of nine of the tumors. A subsequent transcriptome and targeted next-generation sequencing analysis [9,13] revealed alterations (fusions or mutations) within the FGFR1 signaling pathway in all cases with sufficient material (n=8). Six of eight tumors (75%) demonstrated an *FGFR1-TACC1* fusion and two (25%) a hotspot mutation within the kinase domain of *FGFR1* resulting in a c.1638C>A, p.N546K or c.1966A>G, p.K656E substitution (Fig.1 and Supplementary Table 1). The mutant allele frequency for both *FGFR1* mutations was 39%, consistent with a heterozygous somatic mutation. Immunohistochemical detection of phospho-FGFR1 showed diffuse cytoplasmic positivity in all tumors harboring an *FGFR1* alteration (Fig. 2). *FGFR1* encodes the fibroblast growth factor receptor 1, a tyrosine-protein kinase that mainly acts via the MAPK and PI3K pathways. Genetic alterations within the FGFR signaling pathway are common in LGGs and LGGNTs, with missense mutations in *FGFR1* and kinase domain duplications being the most frequent events [4,7,8,15]. In particular, this includes entities such as pilocytic astrocytoma [4], dysembryoplastic neuroepithelial tumor [8], rosette-forming glioneuronal tumor [10] and extraventricular neurocytoma (where fusions are more common) [11]. In addition, *FGFR1* alterations are occasionally found in glioblastoma [12]. Aberrant activation of the FGFR1 pathway is also an attractive molecular target from a therapeutic perspective, since highly potent FGFR inhibitors are currently in clinical trials [5]. These novel treatment options could be of particular value in patients with subtotally resected or diffusely infiltrating tumors, with indication for further therapy. Interestingly, one of the *FGFR1*-mutant tumors also harbored two *NF1* alterations, resulting in c.7006delG, p.A2336fs and c.C2293T, p.R765C. While the frameshift event is likely to be pathogenic, the biological relevance of the missense mutation remains uncertain. Germline material was not available from this patient. Although there are reports of concomitant mutations in LGGs and LGGNTs with potentially overlapping effects on the MAPK/ERK pathway, the vast majority of these tumors typically have only one pathway alteration [2,15]. Besides *FGFR1* and *NF1* alterations, no additional pathogenic mutations were detected (particularly no *BRAF*, *IDH1/2*, *H3*, *ATRX*, *TP53*, *CIC*, *FUBP1* or *TERT* promoter mutations), and none of the tumors harbored a 1p/19q codeletion (Supplementary Table 1).

Most of the tumors were located within the posterior fossa, with exceptions arising in the temporal lobe and in the mesencephalon. Median age at presentation was nine years (range

1–31; 8 of 9 patients younger than 18 years) and the sex distribution was not significantly biased considering the small number of patients (male:female ratio 1.25:1; Fig. 1 and Supplementary Table 1). Treatment and outcome data were unfortunately not available for the majority of the cohort, and further studies will be needed to investigate any prognostic significance of this molecular constellation.

The original histological diagnosis for the majority of the tumors (n=7) was pilocytic astrocytoma World Health Organization (WHO) grade I, with single cases diagnosed as LGG or LGGNT (Fig. 1). In light of their common molecular features, we also reviewed all cases histologically.

All tumors were characterized by a moderate to high cellularity of monomorphic or slightly pleomorphic neoplastic cells with round and partly hyperchromatic nuclei. Most (n=8) harbored areas exhibiting an extensive or focal oligodendroglial morphology with perinuclear halos (Fig. 2 and Supplementary Table 2). Multinucleated cells with nuclei arranged in a 'pennies on a plate' configuration [6] were seen in only one of the tumors. In three cases, tumor cells were focally arranged in ribbons. A microcystic architecture was seen in all cases. Most of the tumors demonstrated either eosinophilic granular bodies (n=6) or Rosenthal fibers (n=3). Extensive calcification was seen in a small number of tumors (n=3). Focal capillary proliferation was observed in five of the cases. Necrosis was uniformly absent. Mitotic activity was very low to absent. Ki-67 labeling activity was 1-2% in seven tumors, while two tumors had a focally elevated proliferation index of up to 5%. All tumors exhibited immunohistochemical expression of glial fibrillary acidic protein (GFAP). Five tumors displayed a weak cytoplasmatic immunoreactivity for synaptophysin. Microtubule-Associated Protein 2 (MAP2) positivity was focally detected in six tumors. Expression of NeuN was entirely negative in all tumors. Altogether, the histological features of all tumors could be considered to be broadly consistent with current descriptions of the morphological spectrum of pilocytic astrocytoma [6,14].

In summary, we have identified a molecularly distinct subset of low-grade glial tumors by DNA methylation profiling with recurrent alterations affecting *FGFR1*, which most commonly arises in the cerebellum and which is histologically enriched for the presence of oligodendroglial morphology in a pilocytic astrocytoma-like background. The prominent cerebellar location and initial diagnosis as pilocytic astrocytoma in most cases suggests that this could represent an FGFR-activated subset of (cerebellar) pilocytic astrocytoma, in contrast to the *KIAA1549-BRAF* fusion that is by far the most commonly observed alteration in this context. Furthermore, the prominent oligodendroglial component of these tumors suggests that they may account for a proportion of tumors still occasionally considered as 'pediatric-type oligodendroglioma'. These findings further support the utility of *FGFR1* molecular testing as part of the diagnostic assessment of pediatric low-grade glial or glioneuronal tumors and identifies a tumor subset with a potential therapeutic target that may be of interest in a subset of patients with sub-totally resected, progressive and/or disseminated disease.

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Figure legends

Fig. 1 Molecular classification of posterior fossa pilocytic astrocytomas with oligodendroglial features (PA PF FGFR1) by DNA Methylation Profiling.

a) Unsupervised hierarchical clustering of DNA methylation profiles of nine PA PF FGFR1 alongside 130 well-characterized glial and glioneuronal reference samples and control tissue using the 20,000 most variably methylated probes. Reference methylation classes: posterior fossa pilocytic astrocytoma (LGG, PA PF), polymorphous low-grade neuroepithelial tumor of the young (PLNTY), ganglioglioma (LGG, GG), midline pilocytic astrocytoma (LGG, PA MID), supratentorial/hemispheric pilocytic astrocytoma and ganglioglioma (LGG, PA/GG), diffuse leptomeningeal glioneuronal tumor subgroup 1 (DLGNT_1), diffuse leptomeningeal glioneuronal tumor subgroup 2 (DLGNT_2), low-grade glioma with MYB or MYBL1 rearrangement (LGG_MYB), extraventricular neurocytoma (EVN), dysembryoplastic neuroepithelial tumor (DNT), rosette-forming glioneuronal tumor (RGNT), papillary glioneuronal tumor (PGNT), central neurocytoma (CN) and control tissue white matter (CONTROL).

b) Two-dimensional representation of pairwise sample correlations using the 15,000 most variant probes by t-distributed stochastic neighbor embedding (t-SNE) dimensionality reduction (same samples as in a).

c) Schematic fusion configuration of case 8 showing an *FGFR1-TACC1* fusion.

d) Clinicopathological characteristics and key genetic alterations identified in the PA PF FGFR1 cohort. Abbreviations: PA, Pilocytic astrocytoma WHO grade I; GTA, Glial tumor with features of anaplasia; LGGNT, Low-grade glioneuronal tumor.

Fig. 2 Morphological and immunohistochemical features of selected tumors from the PA PF FGFR1 cohort. Monomorphic cells with an oligodendroglial morphology (a), expression of FGFR1 in PA PF FGFR1 (b) and control tissue (c).

Supplementary Fig. 1 Copy-number profile derived from DNA methylation array of the *FGFR1*-mutant tumor harboring a concomitant mutation in *NF1*.

Supplementary Fig. 2 Morphological and immunohistochemical features seen in the PA PF FGFR1 cohort. Hematoxylin and eosin (HE)-stained sections show monomorphic cells with round uniform nuclei with perinuclear halos resembling oligodendroglioma (a, b). A subset of tumors is focally arranged in ribbons (c). Typical biphasic pattern of pilocytic astrocytoma with a loose, myxoid component and a more compact component (d) showing Rosenthal fibres and eosinophilic granular bodies (e). Calcifications (f). Bipolar cells with long, narrow processes (g) and multinucleated cells with nuclei arranged in a ‘pennies on a plate’ configuration (h). Glomeruloid microvascular proliferations (i). Immunohistochemically, tumors are GFAP-positive (j) whereas MAP2 (k) and synaptophysin (l) expression is observed only in single cases. Scale bars 100 µm.