Acta Neuropathologica (2021) 142:595–599
https://doi.org/10.1007/s00401-021-02352-w

CORRESPONDENCE

Low-grade glioneuronal tumors with *FGFR2* fusion resolve into a single epigenetic group corresponding to 'Polymorphous low-grade neuroepithelial tumor of the young'

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Received: 2 June 2021 / Revised: 20 July 2021 / Accepted: 21 July 2021 / Published online: 28 July 2021

Low-grade neuroepithelial tumors (LGNET) are a diverse group of neoplasms occurring most commonly in children and young adults, often associated with epilepsy and favorable clinical outcomes. They are composed of a spectrum of tumor entities with divergent clinicopathologic features including gangliocytoma, pilocytic astrocytoma, dysembryoplastic neuroepithelial tumor (DNT), rosette-forming glioneuronal tumor (RGNT), extraventricular neurocytoma (EVN), multinodular and vacuolating neuronal tumor (MVNT), polymorphous low-grade neuroepithelial tumor of the young (PLTNY), myxoid glioneuronal tumor (MGNT), diffuse leptomeningeal glioneuronal tumor (DLGNT), and papillary glioneuronal tumor (PGNT). However, histologically distinguishing between these different LGNET subtypes can be challenging, and molecular profiling is now recognized as critical for accurate classification. While some LGNET subtypes are defined by unique genetic alterations (*e.g. PRKCA* fusion in PGNT [4], *PDGFRA* p.K385L/I dinucleotide mutation in MGNT [9]) that can be used for definitive subtyping, other alterations such as *BRAF* mutation or fusion are nonspecific and can be seen in gangliocytoma, pilocytic astrocytoma, MVNT, and DLGNT [3, 10–12, 14]. *FGFR1* is another promiscuous oncogene in LGNET with kinase domain tandem duplication, gene fusions (most often with *TACC1* as the fusion partner), or hotspot missense mutations at one of two codons within the tyrosine kinase domain (p.N546 or p.K656) recurrently found in pilocytic astrocytoma, DNT, RGNT, and EVN [8, 12–17, 20]. Thus, additional ancillary methodologies such as DNA methylation profiling may be necessary for accurate classification of LGNET with either *BRAF* or *FGFR1* alterations.

Fusions involving the related *FGFR2* oncogene have recently been described as one of the characteristic genetic alterations in the newly recognized tumor entity PLNTY, an epileptogenic neoplasm predominantly occurring in the cerebral hemispheres of children and young adults with oligodendroglioma-like components, abundant calcification, and aberrant CD34 expression [5]. However, rare cases of histologically-defined gangliocytoma, MVNT, DNT, oligodendroglioma, and unclassifiable low-grade glioneuronal tumors have also been reported with *FGFR2* fusions [6, 10, 11, 14]. To improve classification for such *FGFR2*-fused tumors, we performed targeted next-generation sequencing and genome-wide DNA methylation

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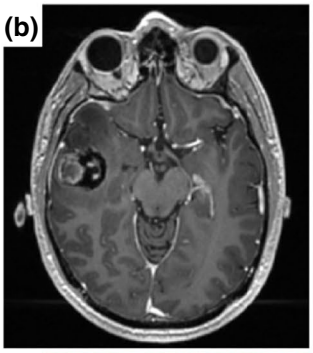
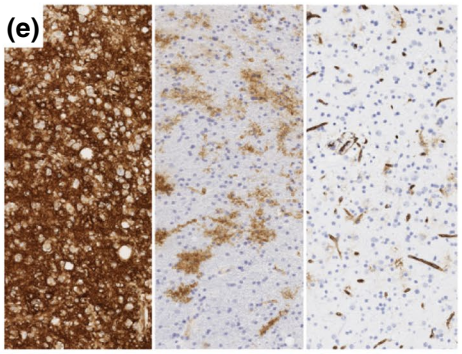
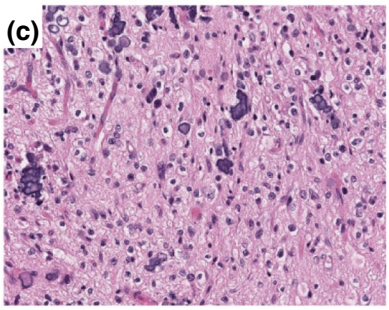
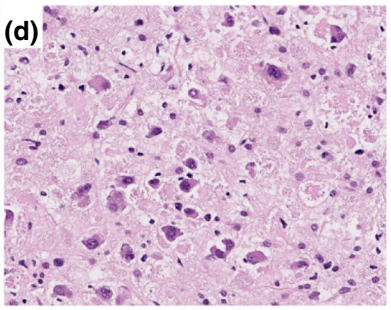
(a)

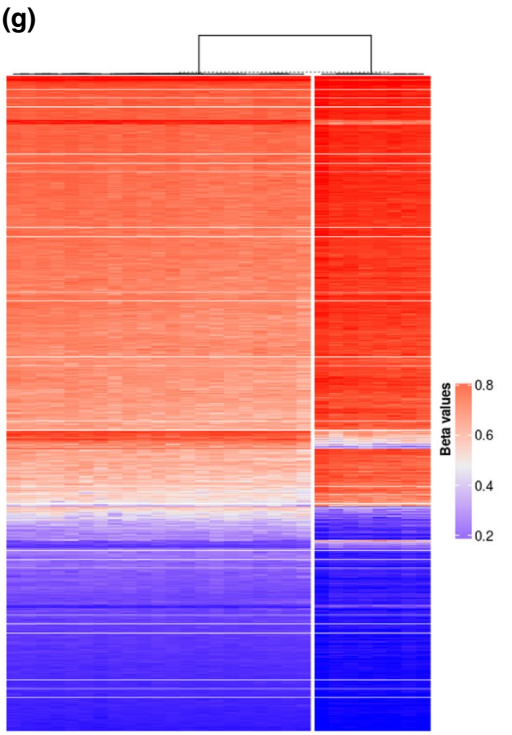
Pt #	Age at dx (yrs)	Sex	Clinical presentation	Location	Histologic diagnosis	*FGFR2* alteration	Methylation class	Previously published
1	11	M	Chronic seizures	R frontal	PLNTY	*FGFR2* rearrangement	not assessed	no
2	17	M	Chronic seizures	L temporal	PLNTY	*FGFR2-ACTR1A* fusion	FGFR2-fused LGNET	no
3	12	M	Chronic seizures	R temporal	mixed MVNT/Ganglioglioma	*FGFR2-INA* fusion	FGFR2-fused LGNET	ref. 10, pt #8
4	10	F	Chronic seizures	L temporal	PLNTY	*FGFR2-INA* fusion	FGFR2-fused LGNET	no
5	7	M	Chronic seizures	R temporal	Ganglioglioma	*FGFR2-KIAA1598* fusion	FGFR2-fused LGNET	ref. 11, pt #34
6	35	M	Chronic seizures	L occipital	Ganglioglioma	*FGFR2-INA* fusion	FGFR2-fused LGNET	ref. 11, pt #35
7	38	M	Seizure	R temporal	PLNTY	*FGFR2-INA* fusion	FGFR2-fused LGNET	no
8	6	F	Chronic seizures	R parietal	LGNET, NOS (favor DNT)	*FGFR2-OPTN* fusion	FGFR2-fused LGNET	no
9	11	F	Chronic seizures	R temporal	PLNTY	*FGFR2* rearrangement	FGFR2-fused LGNET	no

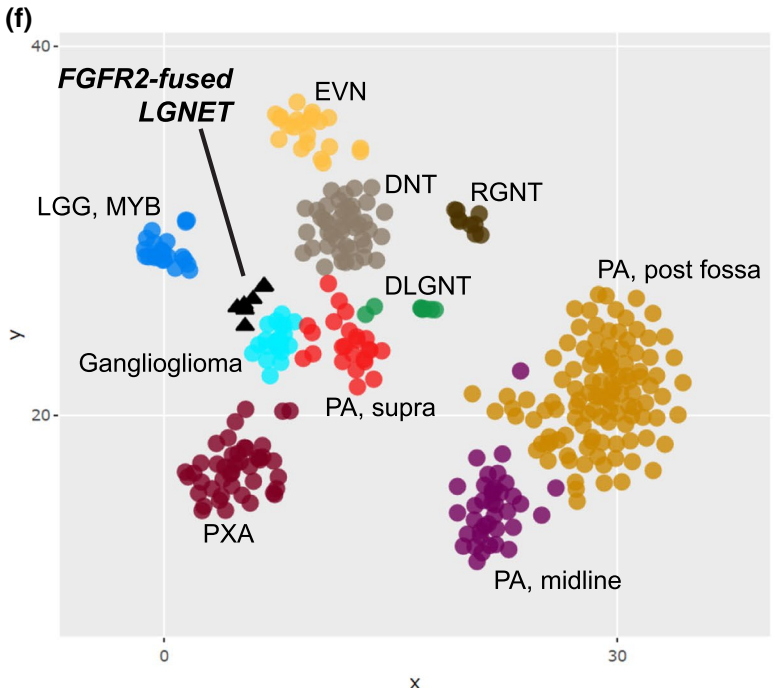


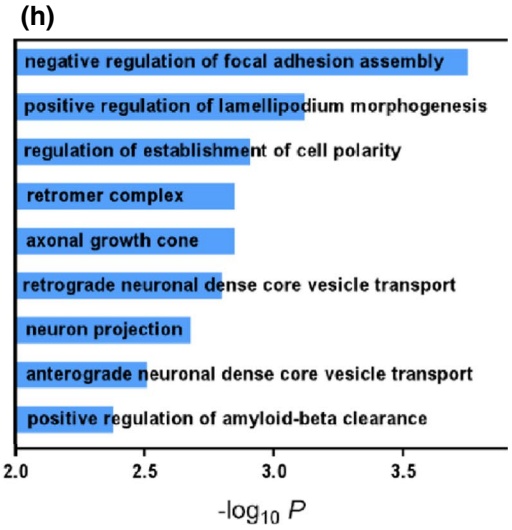












◂**Fig. 1** Clinicopathologic features and epigenomic profiling of low-grade neuroepithelial tumors harboring *FGFR2* gene fusions. **a** Summary table of the 9 patients with *FGFR2*-fused LGNET. **b** MR imaging from patient #7 demonstrating a solid and cystic lesion within the right temporal lobe of the brain. **c** Histology from patient #1 showing characteristic features of PLNTY including round oligodendrocyte-like tumor cells with extensive calcifications. **d** Histology from patient #5 showing numerous dysmorphic ganglion cells and eosinophilic granular bodies without calcifications. **e** Immunochemical staining for CD34 protein showing variable staining patterns in the *FGFR2*-fused LGNET, including diffuse strong staining of tumor cells (left, patient #3), abundant ramified cells (middle, patient #6), and minimal extravascular positivity (right, patient #8). **f** tSNE dimensionality reduction plot of genome-wide DNA methylation profiles from 8 *FGFR2*-fused LGNET alongside 346 reference CNS tumors spanning 10 LGNET entities. **g** Unsupervised hierarchical clustering of DNA methylation data showing segregation of the 8 *FGFR2*-fused LGNET from a reference cohort of 21 gangliogliomas. **h** Differential methylation-based Gene Ontology analysis for *FGFR2*-fused LGNET compared to ganglioglioma, represented in a bar plot of $-\log_{10}$ P values for the most differentially methylated gene networks

profiling on a cohort of 9 patients with LGNET harboring *FGFR2* fusions with a diverse range of histologic diagnoses. Three patients had been previously included in our investigations on the genomic landscape of ganglioglioma and MVNT [10, 11]. The cohort consisted of 6 males and 3 females with median age of 11 years (range 6–38 years), all presenting with chronic seizures (Fig. 1a). Imaging revealed solid and cystic lesions within the cerebral hemispheres (Fig. 1b; Supplementary Fig. 1 [Online Resource 1]). Most patients had gross total resection associated with resolution of seizures and freedom from recurrence during the period of clinical follow-up without adjuvant therapy (Supplementary Table 1 [Online Resource 2]; Supplementary Fig. 2 [Online Resource 1]). Histologically, these were low-grade infiltrative gliomas composed of tumor cells with predominantly round oligodendrocyte-like nuclei (Fig. 1c; Supplementary Fig. 3 [Online Resource 1]; Supplementary Table 2 [Online Resource 2]). Calcifications, often extensive, were present in most but not all tumors. A subset of tumors demonstrated a dysmorphic ganglion cell component and eosinophilic granular bodies (Fig. 1d). Hemosiderin-laden macrophages, indicative of prior intra-tumoral hemorrhage, were also a common finding. One tumor demonstrated vacuolation in both the stroma and ganglion cell component resembling MVNT. Rosenthal fibers, necrosis, and microvascular proliferation were not encountered. Mitotic activity was inconspicuous, and Ki67 labeling was less than 2% in all examined tumors. Neurofilament and synaptophysin staining revealed entrapped axonal processes in the background of all tumors, and additionally highlighted the dysmorphic ganglion cell component in a subset (Supplementary Table 3 [Online Resource 2]). Immunohistochemistry for CD34 demonstrated diffuse strong labeling of tumor cells characteristic of PLNTY in 6 of 9 tumors, and showed only scattered ramified cells more characteristic of ganglioglioma in the other 3 tumors (Fig. 1e). The original histopathologic diagnosis was PLNTY ($n=5$), ganglioglioma ($n=2$), mixed MVNT/ganglioglioma ($n=1$), and unclassifiable LGNET ($n=1$). Four tumors demonstrated *FGFR2* fusion with *INA* as the partner, one tumor each demonstrated fusion with *KIAA1598*, *ACTR1A*, and *OPTN*, and two tumors demonstrated complex *FGFR2* rearrangements with uncertain fusion partner based on the targeted DNA sequencing analysis (Supplementary Table 4 [Online Resource 2]). The *FGFR2* fusion was the solitary pathogenic alteration identified in all tumors, with an absence of accompanying alterations involving *IDH1/2*, histone H3 genes, *BRAF*, *NF1*, *PRKCA*, *FGFR1*, *PIK3CA*, *PIK3R1*, *PTEN*, *CDKN2A*, *TP53*, *TERT* (including promoter region), *ATRX*, *CIC*, *FUBP1*, *MYB*, and *MYBL1* [7]. The quantity of chromosomal copy number aberrations was variable, but no tumors harbored whole arm co-deletion of chromosomes 1p and 19q, nor were there focal amplifications or homozygous deletions in any tumors (Supplementary Table 5 [Online Resource 2]).

Genome-wide DNA methylation profiling was performed on 8 of the tumors using Infinium EPIC 850k Beadchips (Illumina) following the manufacturer's recommended protocols (see Supplementary Methods [Online Resource 3]). tSNE clustering of the DNA methylation data alongside reference cohorts of CNS tumors revealed that the *FGFR2*-fused LGNET formed a single epigenetic group that was distinct from all methylation classes in the current version of the DKFZ classifier v11b4 (Fig. 1f; Supplementary Fig. 4 [Online Resource 1]; Supplementary Tables 6–7 [Online Resource 2]) [2]. Unsupervised hierarchical clustering successfully segregated the 8 *FGFR2*-fused LGNET from a reference cohort of 21 gangliogliomas (Fig. 1g). Gene Ontology analysis of the most differentially methylated gene regions between *FGFR2*-fused LGNET and ganglioglioma revealed gene networks involved in cell polarity, lamellipodium morphogenesis, and neuronal functions including growth cone projection and core vesicle transport (Fig. 1h; Supplementary Tables 8–10 [Online Resource 2]). tSNE dimensionality reduction of the DNA methylation data alongside 3 histologically-defined cases of PLNTY with *FGFR2* fusion from Huse et al. [5] demonstrated close clustering, indicating low-grade glioneuronal tumors with *FGFR2* fusion resolve into a single epigenetic group (Supplementary Fig. 5 [Online Resource 1]). Additionally, tSNE dimensionality reduction and unsupervised hierarchical clustering of 3 histologically-defined cases of PLNTY with *BRAF* p.V600E mutation from Huse et al. [5] demonstrated overall epigenetic similarity to *FGFR2*-fused LGNET but resolved into a separate methylation subgroup by both

methodologies, suggesting that PLNTY may be composed of at least two distinct epigenetic subgroups—those with *FGFR2* fusion and those with *BRAF* p.V600E mutation (Supplementary Fig. 5 and 6 [Online Resource 1]).

Here, we demonstrate LGNET with *FGFR2* fusions exhibit a spectrum of histologic features, but share an epigenetic signature distinct from all of the reference LGNET methylation classes in the current version 11b4 of the DKFZ classifier. Although most *FGFR2*-fused LGNET belonging to this unique methylation class have histologic features aligning with PLNTY, a subset is devoid of calcifications, have CD34 positivity limited to scattered ramified cells, and contain a prominent ganglion cell component, thereby complicating their differentiation from gangliolioma based on microscopic features alone. Furthermore, while *BRAF* and *FGFR1* alterations are promiscuous and recurrently present across various LGNET types, *FGFR2* fusions among LGNET appear to be quite specific to this distinct epigenetic subgroup of glioneuronal tumors. Notably, similar *FGFR2* gene fusions are frequent in intrahepatic cholangiocarcinoma for which clinical trials have shown promising efficacy of small molecule tyrosine kinase inhibitors such as erdafitinib [1, 19]. It remains unclear why *FGFR2* fusions are selected for in PLNTY and intrahepatic cholangiocarcinoma, whereas *FGFR1* alterations are selected for in pilocytic astrocytoma, DNT, RGNT, and EVN, but is likely to reflect differences in response to the ligand-binding specificity for the various fibroblast growth factors [18]. Future studies are required to define the full clinicopathologic spectrum of *FGFR2*-fused LGNET and the potential efficacy of genomically tailored therapy for affected patients.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00401-021-02352-w.

Acknowledgements We thank Jason Huse, Matija Snuderl, David Jones, and Marc Rosenblum for sharing the DNA methylation array data from their previously published PLNTY cohort [5]. C.G.L. is supported by the UCSF Training Program in Translational Brain Tumor Research, National Cancer Institute, National Institutes of Health (T32 CA151022). This study was supported by the NIH Director's Early Independence Award from the Office of the Director, National Institutes of Health (DP5 OD021403) to D.A.S.; a Developmental Research Program Award from the UCSF Brain Tumor SPORE from the National Cancer Institute, National Institutes of Health (P50 CA097257) to D.A.S.; a generous gift from the Morgan Adams Foundation to D.A.S.; a generous gift from the Yuvann Tiwari Foundation to D.A.S.; and the UCSF Glioblastoma Precision Medicine Program sponsored by the Sandler Foundation. We thank the staff of the UCSF Clinical Cancer Genomics Laboratory for assistance with genetic profiling.

Data availability Scanned image files of H&E and CD34 stained sections from the 9 tumors in this cohort are available for downloading and viewing at the following link: https://figshare.com/projects/Low-grade_neuroepithelial_tumors_with_FGFR2_fusion/113418. DNA methylation array data files from this study are available from the Gene Expression Omnibus (GEO) repository under accession number GSE172081 (https://www.ncbi.nlm.nih.gov/geo/). Structural variant and copy number data are available in the electronic supplementary material. Raw sequencing data files are available upon request.

Declarations

Conflict of interest The authors declare that they have no competing interests related to this report.

Ethical approval This study was approved by the Committee on Human Research of the University of California, San Francisco, with a waiver of patient consent.

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