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Original contribution

Clinicopathologic implications of *NF1* gene alterations in diffuse gliomas☆

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Summary Recent studies have identified somatic alterations in the gene encoding for neurofibromin (*NF1*) in a subset of glioblastoma (GBM), usually associated with the mesenchymal molecular subtype. To understand the significance of *NF1* genetic alterations in diffuse gliomas in general, we evaluated public databases and tested for *NF1* copy number alterations in a cohort using fluorescence in situ hybridization. *NF1* genetic loss (homozygous *NF1* deletions or mutations with predicted functional consequences) was present in 30 (of 281) (11%) GBM and 21 (of 286) (7%) lower-grade gliomas in The Cancer Genome Atlas data. Furthermore, *NF1* loss was associated with worse overall and disease-specific survival in the lower-grade glioma, but not GBM, Group in The Cancer Genome Atlas cohort. *IDH1* or *2* mutations co-existed in lower-grade gliomas with *NF1* loss (36%) but not in GBM. In our cohort studied by fluorescence in situ hybridization, *NF1*/17q (n = 2) or whole Ch17 (n = 3) losses were only identified in the GBM group (5/86 [6%]). Tumors with *NF1*/Ch17 loss were predominantly adult GBM (4/5); lacked *EGFR* amplification (0/4), strong p53 immunolabeling (1/5), or IDH1 (R132H) protein expression (0/5); but expressed the mesenchymal marker podoplanin in 4/5. *NF1* genetic loss occurs in a subset of diffuse gliomas, and its significance deserves further exploration.

1. Introduction

Neurofibromatosis type 1 (NF1) is a genetic tumor-predisposing syndrome caused by germline mutations in the *NF1* gene and is inherited in an autosomal dominant fashion [1,2]. The *NF1* gene, located at chromosome region 17q11.2, encodes neurofibromin, a tumor suppressor that works primarily by regulating RAS but also modulates adenyl cyclase [3,4]. *NF1* loss generally leads to increased activity in a variety of pro-tumorigenic pathways, particularly the mitogen-activated protein kinase pathway (Fig. 1). Patients with NF1 are predisposed to a variety of tumors affecting the central and peripheral nervous system. In the central nervous system, the predominant tumor type is pilocytic astrocytoma, which in this patient population has a propensity to involve the optic pathways. However, NF1 patients, especially adults, may develop gliomas of all types and grades [5]. In a large retrospective study of NF1-associated gliomas classified using current World Health Organization (WHO) criteria, 27% were diffusely infiltrating astrocytomas, and 22% were high grade (ie, anaplastic astrocytomas and glioblastomas) [6].

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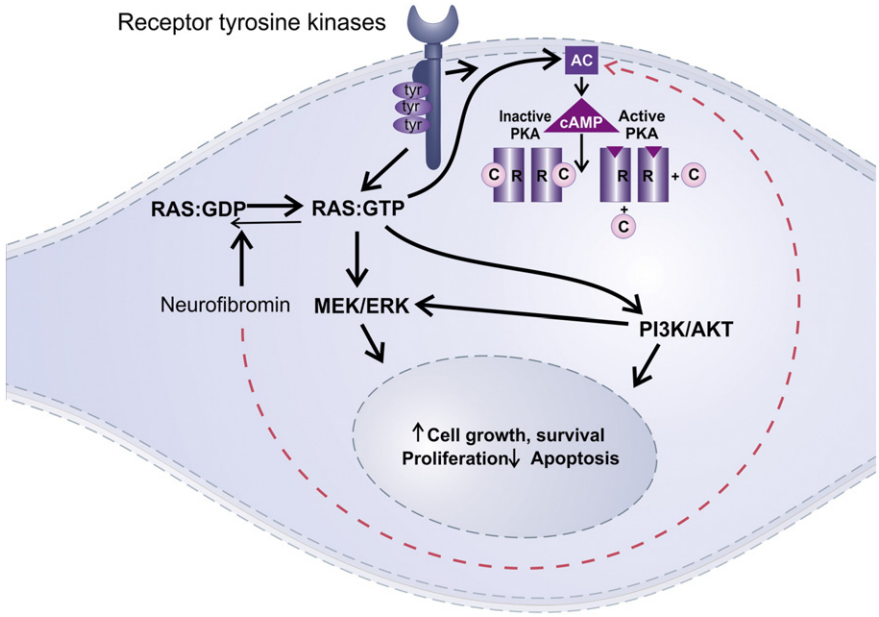

Fig. 1 Cellular pathways affected by neurofibromin loss. Neurofibromin is an important tumor suppressor that negatively regulates RAS by driving it to the GDP bound state. Neurofibromin loss therefore leads to constitutive RAS activation and MEK/ERK signaling, resulting in pro-tumorigenic signals. Neurofibromin also has a lesser known function acting through adenyl cyclase (AC) (dotted arrow).

A role for *NF1* loss in sporadic astrocytomas has also been elucidated more recently. Sporadic pilocytic astrocytomas do not have alterations in *NF1* [7], but rather *BRAF* fusions or mutations [8–11], as well as activating mutations in other oncogenes [12,13]. However, early clues that alterations in the *NF1* gene may be important in the development of diffusely infiltrating gliomas were provided by mouse models in which *Nf1* and *Tp53* co-inactivation led to glioblastoma formation with high, if not complete, penetrance [14,15]. More recent large-scale comprehensive analyses have identified somatic alterations in the *NF1* gene in a subset of glioblastoma [16,17], usually associated with the mesenchymal molecular subtype [18].

The mesenchymal molecular subtype was initially identified by gene expression profiling studies of high-grade gliomas that separated proneural, mesenchymal and proliferative classes. The mesenchymal signature defined a group with a relatively worse prognosis and relatively enriched in recurrent tumors, with frequent up-regulation of YKL40, CD44, and STAT3 [19]. Recent analysis of data from lower-grade gliomas (II-III) also highlighted the relevance of the glioblastoma molecular subtypes with respect to biology and prognosis [20]. However, the mesenchymal subclass was almost entirely restricted to glioblastomas in this study.

Germline *NF1* mutations may be of many different types, including mutations altering splicing of mRNA, as well as nonsense, missense, and frameshift mutations [21,22]. Approximately 5% of NF1 patients have constitutional large gene deletions, which may be associated with a more severe phenotype [23,24]. Large constitutional deletions may also have an effect on tumor burden in a subset of patients [25].

At the current moment it is unclear what the role of *NF1* genetic alterations, particularly deletions and mutations with predicted functional effects, is in the sporadic diffuse glioma subtypes. In the current study, we tested for *NF1* copy number alterations in a cohort of diffuse gliomas, evaluated public databases, and searched for associations with pathologic and molecular features.

2. Materials and methods

2.1. The Cancer Genome Atlas data analysis

The cBIOPORTAL for cancer genomics website from Memorial Sloan Kettering Cancer Center (http://www.cbioportal.org/public-portal) was used to analyze The Cancer Genome Atlas (TCGA) data for *NF1* alterations in diffuse gliomas (date of analysis 8 December 2014). Copy number/genotype platforms listed for the glioblastoma dataset are Affymetrix SNP6 and Agilent 224K/415K [26]. Data for homozygous deletions were obtained through the GISTIC algorithm. To exclude non-relevant "passenger" mutations or germline variants, we focused on mutations with predicted functional consequences as specified in cBIOPORTAL using previously published guidelines by Reva et al. [27]. In brief, mutations are assigned a functional impact score based on the extent of conservation of the specific amino acid residues across and within species. "Medium" and "High" scores (>~1.9) are predicted to be functional (ie, relevant to disease). Details of cBIOPORTAL analysis have been previously published [28,29]. Frequencies were compared using the Fisher exact test. Survival analysis was performed using Kaplan-Meier curves and the

log-rank test per the cBIOPORTAL algorithm. In addition, available scanned hematoxylin and eosin (H&E) slides were reviewed and *IDH* mutation status was abstracted in the subset of lower-grade glioma cases with *NF1* alterations in cBIOPORTAL.

2.2. Primary tumor samples

A cohort of 130 diffuse gliomas obtained from two tissue microarrays constructed at Johns Hopkins Hospital were studied, including 86 glioblastomas (57 adults, 29 pediatric) and 44 lower-grade diffuse gliomas (grades II-III). Two to four 0.6 mm diameter cores per tumor were evaluated. Clinicopathologic features of these cases have been previously published [30]. All studies were performed under institutional review board approval and following all guidelines.

2.3. Immunohistochemistry

Immunohistochemical staining was performed in formalin-fixed paraffin-embedded tissue microarray sections using a podoplanin antibody (clone D2-40, DAKO, Carpinteria, CA) 1:200). A biotin-free polymer detection kit was used (Bond Polymer Refine Detection, Leica Microsystems, Bannock Burn, IL). At least 2 TMA scores per case were required for score assignment. Scoring was performed by 2 observers (M.A.V., F.J.R.) using a three-tiered scale based on staining intensity (0-3+) (Supplementary Fig. 1). Non-neoplastic gray and white matter were used as controls.

2.4. Fluorescence in situ hybridization

A custom-made fluorescence in situ hybridization (FISH) probe targeting the *NF1* gene (17q11.2) with a CEP17 probe control was obtained from Empire Genomics (Buffalo, NY), and hybridized using previously described methods [31]. Sections were evaluated using an Olympus PROVIS (Center Valley, PA) fluorescence scope with appropriate filters. At least 50 non-overlapping nuclei were evaluated per case. Heterozygous deletion was defined as a target-to-control ratio of 0.80 or less. Monosomy was defined as loss of target and control probe in at least 60% of cells. Chromosome gain was defined as an extra copy of target and control probe in at least 30% of cells. Scoring and calls were made by an observer blinded as to the specific pathology of the specimens (M.A.V.), and confirmed by a second observer (F.J.R.).

3. Results

3.1. *NF1* mutations and homozygous deletions in glioblastomas and lower-grade gliomas in TCGA

We first looked at genomic data of diffuse gliomas from The Cancer Genome Atlas (TCGA) using the cBIOPORTAL for cancer genomics website from Memorial Sloan Kettering Cancer Center (http://www.cbioportal.org/public-portal) to determine the frequency of *NF1* genetic alterations in diffuse gliomas of various grades (II-III). Genomic alterations (mutations with predicted functional consequences and homozygous deletions) were present in the *NF1* gene in 30 (of 281) (11%) glioblastomas with sequencing and copy number data (homozygous deletion in 5, truncating mutations in 19, missense mutations with predicted functional effects in 4, in-frame heterozygous deletion in 1, and an additional single case with a homozygous deletion and a truncating mutation). A single case had a missense mutation with no predicted functional effects. None of these tumors had *IDH1* or *IDH2* mutations. Survival analysis did not demonstrate any differences when comparing glioblastomas with or without *NF1* alterations (Fig. 2A and B). In the lower-grade glioma group, 21 (of 286) (7%) cases with sequencing and copy number data had inactivating alterations in the *NF1* gene (homozygous deletions in 3, truncating mutations in 15, missense mutations with predicted functional effects in 3). A single case had a missense mutation with no predicted functional effects. Overall and disease-specific survival were worse in lower-grade gliomas with *NF1* alterations compared to those without ($P = .0001$) (Fig. 2C and D).

Histologically, this subgroup of lower-grade gliomas with *NF1* genetic loss included low-grade astrocytoma (WHO grade II) (n = 10), low-grade oligodendroglioma (n = 2), oligoastrocytomas (WHO grade II-III) (n = 7), anaplastic astrocytoma (WHO grade III) (n = 1), and anaplastic oligodendroglioma (WHO grade III) (n = 1) per ICD-O-3-HISTOLOGY codes listed in cBIOPORTAL per case. Permanent H&E slides were available for review in 8 cases and confirmed a predominance of astrocytic histology, including the only 2 oligoastrocytomas with available slides. Of interest *IDH1*/2 mutations co-occurred in 8 (of 21) (38%) of these tumors (*IDH1* [R132H] in 7, *IDH2* [R172K] in 1). Three (of 5) of these patients with tumors with co-existing *NF1* and *IDH1/2* mutations and follow up >1 year were dead 22 to 46 months after diagnosis. *FUBP1* and/or *CIC* mutations, typical of oligodendrogliomas, were present in only three (of 21) of these cases.

When looking at TCGA tumors with copy number alteration data only, *NF1* deletions were present in (7/563 [1.2%]) glioblastomas compared to lower-grade gliomas (4/512 [0.7%]), but this did not achieve statistical significance ($P = .55$, Fisher exact test).

3.2. Large *NF1* copy number alterations occur in a subset of glioblastomas

Next we searched for copy number alterations in a cohort of diffuse gliomas grades II-IV from JHH, in order to confirm our observations above regarding *NF1* genetic loss in public databases. We chose FISH, a technique readily applicable to formalin-fixed, paraffin-embedded tissues and

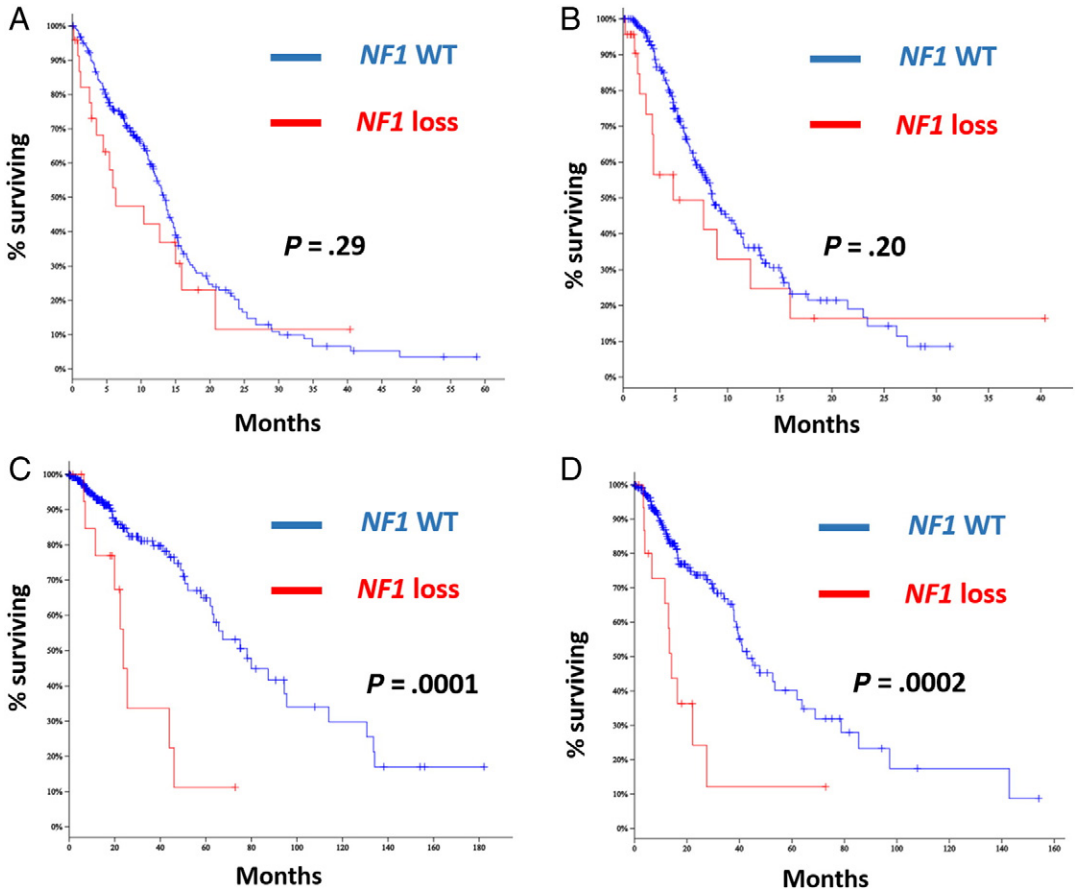

Fig. 2 Overall and disease-specific survival by *NF1* genetic alterations (loss) from the TCGA dataset. *NF1* genetic loss shows no effect in overall (A) or disease-specific survival (B) in glioblastoma. However, in the lower-grade glioma group, *NF1* loss was associated with significantly worse overall (C) and disease-specific survival (D) (log-rank test). (WT = wild type; *NF1* loss includes homozygous deletions and predominantly mutations with functional consequences).

suitable for identification of tumor areas with genetic alterations, even focally. In addition, this technique allows for the identification of large, possibly more deleterious, deletions. *NF1*/17q or Ch 17 gains/polysomies were relatively common and identified in 40 (31%) of 130 cases across tumors tested, including 2 of 11 diffuse astrocytomas, 6/16 anaplastic astrocytomas, 7 of 17 oligodendroglial tumors, and 25 (29%) of 86 glioblastomas (GBM) (8/29 pediatric GBM, 17/57 adult GBM). Of greater functional interest, *NF1*/17q (n = 2) or whole Ch17 (n = 3) losses were only identified in the GBM group (6% [5/86]) (Fig. 3 and Table 1).

3.3. Clinicopathologic and molecular features of glioblastomas with *NF1* losses

When looking at the GBM subset with *NF1*/Ch17 loss in the JHH cohort, the tumors were predominantly adult GBM (4/5). Pathologic examination revealed that 2 of the 5 tumors were gliosarcomas (Table 2). The diagnosis of gliosarcoma was performed using current WHO criteria, specifically demonstrating distinct glial fibrillary acidic Protein (GFAP)-positive, reticulin-poor glial neoplastic areas and GFAP-negative, reticulin-rich sarcomatous areas [32]. Of the remaining 3, one had small cell features, one was fibrillary, and one was gemistocytic. Molecular and immunohistochemical analysis demonstrated that these tumors predominantly lacked *EGFR* amplification (0/4), strong p53 immunolabeling (1/5), mutant IDH1 (R132H) protein expression (0/5), or the alternative lengthening of telomeres phenotype (1/5). Median overall survival was 32 months (range, 10-118) (Tables 1 and 2).

Given the strong association between *NF1* loss and the mesenchymal phenotype in glioblastoma, we next tested the samples with an antibody against podoplanin, which is in broad clinical use and previously used as a marker of the mesenchymal subtype in paraffin tissues [33]. Podoplanin immunoreactivity when present was diffuse and varied primarily in staining intensity, therefore the latter represented the scoring parameter. Glioblastomas with *NF1* loss expressed podoplanin more frequently (4/5) than other diffuse gliomas (45% [55/122]), although this was not statistically significant ($P = .18$), likely related to the small number of cases of interest (Fig. 4). Of note, almost all IDH1 (R132H) mutant tumors (except for two) were podoplanin negative.

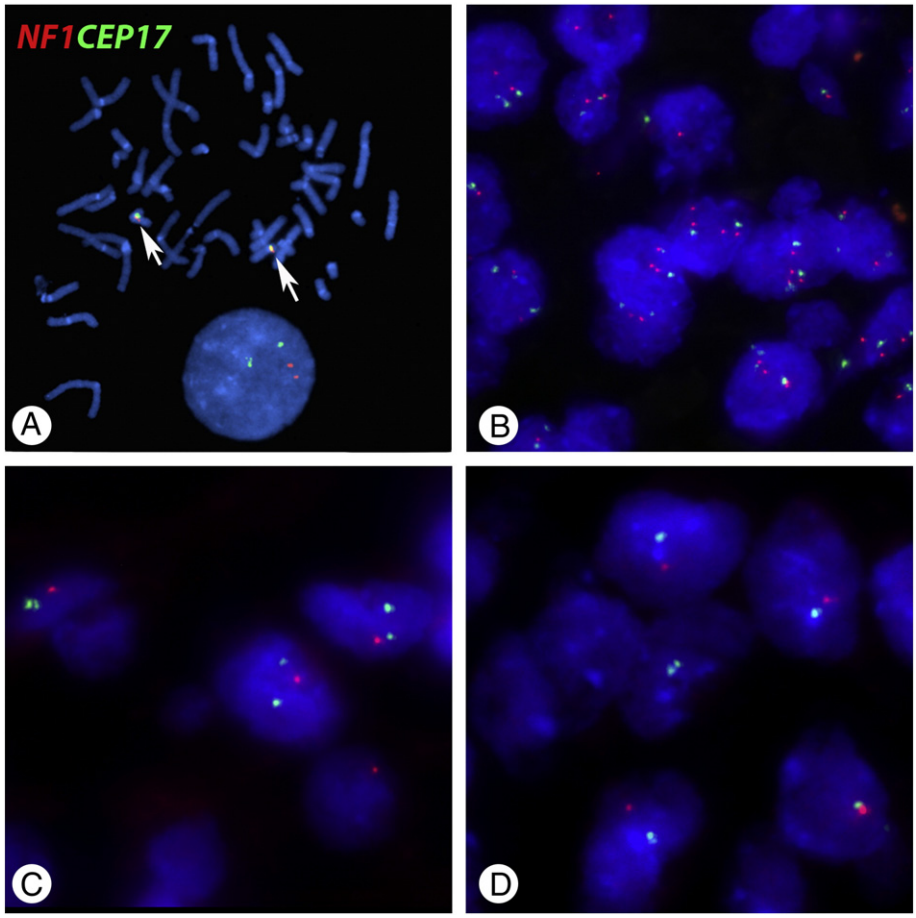

Fig. 3 Copy number alterations in the *NF1* gene in diffuse gliomas. A, Normal human metaphases demonstrate proper targeting of the probes for the *NF1* locus (Orange *5-TAMRA dUTP*) and centromere 17 (Green *5-Fluorescein dUTP*) (arrows) (original magnification ×1000). B, Nuclei are stained blue (DAPI). The most frequent alteration was polysomy involving chromosome 17 (original magnification ×600). Conversely, the more functionally relevant alteration of *NF1*/17q loss (original magnification ×1000) (C) or monosomy 17 (original magnification ×1000)(D) were present in a small subset of glioblastomas.

4. Discussion

The *NF1* gene has emerged in recent years as an important tumor suppressor gene in gliomas, not only in those associated with the syndrome but also those that (more commonly) develop sporadically. Previous analyses of the TCGA dataset in glioblastoma uncovered a strong association between somatic *NF1* mutation and the mesenchymal molecular subtype [18]. The mesenchymal subtype, as the name suggests, was recognized initially by a gene expression signature [19]. Subsequent work demonstrated that this phenotype is driven by a set of transcription factors (C/EBPβ and STAT3), which are able to reprogram neural stem cells along this phenotype [34]. Numerous markers have been proposed as being useful in characterizing the mesenchymal molecular subtype. We chose podoplanin in this study because it is commercially available and routinely used in many laboratories, and has been proposed by some groups as a marker useful in proteomic subclassification of glioblastoma [33], although it has also been found to label reactive glial cells in the context of gliomas and other pathologies [35]. One caveat is that glioblastoma and other diffuse gliomas are increasingly recognized as demonstrating genetic and phenotypic heterogeneity, and the small areas studied in TMAs may not be fully representative of the tumor expression patterns.

Table 1 Pathologic and molecular features of gliomas with NF1/Ch17 loss

	NF1/Ch17loss n = 5	Ch17 intact n = 125
Glioma subtype	GBM 5/5 (100%)	81/125 (65%)
	Adult GBM 4/5	53/125 (42%)
	Pediatric GBM 1/5	28/125 (22%)
	Diffuse (II-III) (0)	44/125 (35%)
Podoplanin IHC	4/5	55/122 (45%)
EGFR amp	0/4	17/90 (19%)
p53 IHC	1/5	32/96 (33%)
IDH1 (R132H) IHC	0/5	37/125 (30%)

Table 2 Clinical, pathological and molecular features of GBM group with *NF1* loss

AGE	Tumor Type	Tumor Location	Histologic subtype	Ch17	PODOPLANIN IHC	EGFR FISH	IDH1 (R132H)	p53 IHC	ALT	Overall survival (Mo)
81	Adult GBM	R temporal lobe	Gemistocytic	Monosomy	Pos	NA	Neg	Neg	Neg	32
47	Adult GBM	R frontal lobe	Fibrillary	Monosomy	Pos	No AMP	Neg	Neg	Neg	118
14	Peds GBM	Cerebellum	Small cell features	*NF1* loss	Neg	No AMP	Neg	Neg	Pos	42
59	Adult GBM	L temporal lobe	Gliosarcoma	Monosomy	Pos	No AMP	Neg	Pos	Neg	21
59	Adult GBM	L parietooccipital	Gliosarcoma	*NF1* loss	Pos	No AMP	Neg	Neg	Neg	10

Abbreviations: R, right; L, left; Ch, chromosome; IHC, immunohistochemistry; Pos, positive; Neg, negative; AMP, amplification; NA, not available.

In this study, we evaluated a cohort of diffuse gliomas for copy number alterations involving the *NF1* gene. Heterozygous deletion of the *NF1* gene region/17q or whole chromosomal loss (monosomy 17) was actually restricted to a small subset of glioblastomas and were completely absent in a group of diffuse gliomas of various types and grades. Although these tumors have relatively distinct molecular genetic features, the histology was variable. Of interest, 2 of the cases with *NF1*/Ch17 loss were histologically gliosarcomas. Prior cytogenetic studies of gliosarcoma have demonstrated a similar genetic profile to glioblastoma, including 7,X,9q, and 20q gain, as well as 9p, Ch10, and 13q loss [36,37], although 17q loss does not appear to be particularly common.

Also of interest is that the survival of the patients with the *NF1*-deleted group was not necessarily poor, although this alteration was strictly limited to the glioblastoma group. Despite the expression of podoplanin by 4 (of 5) of these cases, the relatively long survival of some of these cases raises the possibility that these tumors are not all of the mesenchymal subtype. This subtype has been previously associated with a high frequency of somatic *NF1* loss, although the alterations identifiable in the TCGA dataset include point mutations and small deletions. It is unclear if large deletions identifiable by FISH or whole chromosomal loss has the same significance but may identify a distinct subset of glioblastoma. Of interest, two of the tumors in our dataset with *NF1* loss were gliosarcomas, and the survival of these 2 patients was shorter (10 and 21 months).

When looking at data from the TCGA, deletions involving the *NF1* gene were relatively rare, although given the higher resolution of the platforms employed, only homozygous deletions were studied. They were present in both the glioblastoma and lower-grade glioma group.

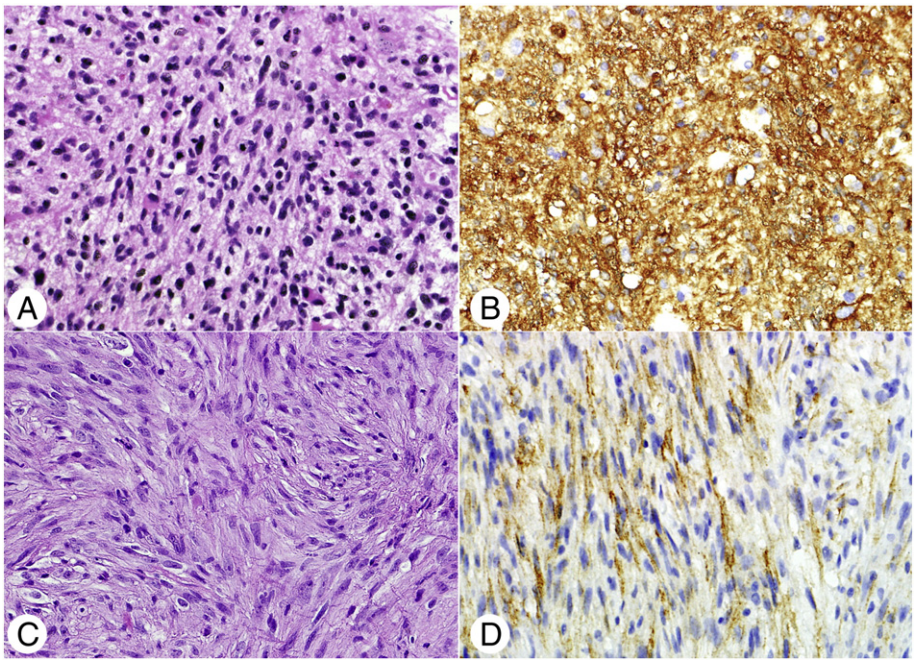

Fig. 4 Histologic and immunohistochemical features of diffuse gliomas with *NF1* loss. The histology of the 5 tumors with *NF1* loss was variable, including fibrillary astrocytoma (H&E; original magnification ×400) (A) and 2 gliosarcomas The latter were characterized by neoplastic spindle cells (C), lacking GFAP expression and demonstrating pericellular reticulin staining (not shown). Matched podoplanin immunohistochemical stains demonstrating strong (B) and more modest (D) overexpression in these tumors (original magnification ×400).

Although slightly more frequent in glioblastoma, the difference was not statistically significant. This contrasts with the absence of *NF1* genetic loss in our cohort of lower grade diffuse gliomas using FISH. This discrepancy could be explained in part by the fact that large *NF1* deletions identifiable by FISH reflect increased genomic instability in higher grade gliomas, that is, glioblastoma, and that smaller *NF1* deletions present in lower-grade gliomas require platforms with higher resolution using frozen tissue, such as those used by the TCGA.

A clinically important finding when looking at the TCGA data is that *NF1* loss (homozygous deletions or predicted inactivating mutations) was associated with worse survival in the lower-grade glioma group. Given the correlation of *NF1* loss with the mesenchymal molecular subtype in glioblastoma, it is possible that sporadic lower-grade gliomas with *NF1* loss represent a group more similar to primary glioblastoma than other lower-grade gliomas. The predominant histologic type in this group was diffuse astrocytoma (WHO grade II), which suggests that histologic type (but probably not grade) may account for some of these survival differences. In fact, only 3 pure oligodendrogliomas were present in this group, and *CIC* and *FUBP1* mutations, typical of oligodendrogliomas [38], were absent in the latter. Of interest, *IDH1/2* mutations occurred in approximately a third of these lower-grade gliomas with *NF1* loss, but not in glioblastomas, albeit at a lower frequency than the lower-grade glioma group as a whole (~80%). This suggests that the effects of *NF1* loss on this group cannot be ascribed completely to early glioblastomas, at the pathologic or molecular level, but that it may have additional prognostic implications. However, characterization of the full clinical and biologic significance of *NF1* genetic loss in diffuse gliomas, and its cooperation with other genetic alterations, will require further studies.

In summary, *NF1*/Ch17 gains occur in a subset of diffuse gliomas, irrespective of grade and pathologic subtype. Conversely, *NF1*/Ch17 loss identifiable by FISH is restricted to a small GBM subset, a finding that deserves further exploration. In TCGA datasets *NF1* alterations occur in both GBM and lower-grade diffuse gliomas and may be associated with worse outcome in the latter group, which has prognostic implications.

Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.humpath.2015.05.014.

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References

[1] Cawthon RM, Weiss R, Xu GF, et al. A major segment of the neurofibromatosis type 1 gene: cDNA sequence, genomic structure, and point mutations. Cell 1990;62:193-201.
[2] Wallace MR, Marchuk DA, Andersen LB, et al. Type 1 neurofibromatosis gene: identification of a large transcript disrupted in three NF1 patients. Science 1990;249:181-6.
[3] Guo HF, Tong J, Hannan F, Luo L, Zhong Y. A neurofibromatosis-1-regulated pathway is required for learning in *Drosophila*. Nature 2000;403:895-8.
[4] Carroll SL. Molecular mechanisms promoting the pathogenesis of Schwann cell neoplasms. Acta Neuropathol 2012;123:321-48.
[5] Gutmann DH, James CD, Poyhonen M, et al. Molecular analysis of astrocytomas presenting after age 10 in individuals with NF1. Neurology 2003;61:1397-400.
[6] Rodriguez FJ, Perry A, Gutmann DH, et al. Gliomas in neurofibromatosis type 1: a clinicopathologic study of 100 patients. J Neuropathol Exp Neurol 2008;67:240-9.
[7] Kluwe L, Hagel C, Tatagiba M, et al. Loss of NF1 alleles distinguish sporadic from NF1-associated pilocytic astrocytomas. J Neuropathol Exp Neurol 2001;60:917-20.
[8] Sievert AJ, Jackson EM, Gai X, et al. Duplication of 7q34 in pediatric low-grade astrocytomas detected by high-density single-nucleotide polymorphism-based genotype arrays results in a novel BRAF fusion gene. Brain Pathol 2009;19:449-58.
[9] Jones DT, Kocialkowski S, Liu L, et al. Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas. Cancer Res 2008;68:8673-7.
[10] Pfister S, Janzarik WG, Remke M, et al. BRAF gene duplication constitutes a mechanism of MAPK pathway activation in low-grade astrocytomas. J Clin Invest 2008;118:1739-49.
[11] Bar EE, Lin A, Tihan T, Burger PC, Eberhart CG. Frequent gains at chromosome 7q34 involving BRAF in pilocytic astrocytoma. J Neuropathol Exp Neurol 2008;67:878-87.
[12] Jones DT, Hutter B, Jager N, et al. Recurrent somatic alterations of FGFR1 and NTRK2 in pilocytic astrocytoma. Nat Genet 2013;45:927-32.
[13] Zhang J, Wu G, Miller CP, et al. Whole-genome sequencing identifies genetic alterations in pediatric low-grade gliomas. Nat Genet 2013;45:602-12.
[14] Reilly KM, Loisel DA, Bronson RT, McLaughlin ME, Jacks T. Nf1;Trp53 mutant mice develop glioblastoma with evidence of strain-specific effects. Nat Genet 2000;26:109-13.
[15] Zhu Y, Guignard F, Zhao D, et al. Early inactivation of p53 tumor suppressor gene cooperating with NF1 loss induces malignant astrocytoma. Cancer Cell 2005;8:119-30.
[16] Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 2008;455:1061-8.
[17] Parsons DW, Jones S, Zhang X, et al. An integrated genomic analysis of human glioblastoma multiforme. Science 2008;321:1807-12.
[18] Verhaak RG, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 2010;17:98-110.
[19] Phillips HS, Kharbanda S, Chen R, et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell 2006;9:157-73.

[20] Guan X, Vengoechea J, Zheng S, et al. Molecular subtypes of glioblastoma are relevant to lower grade glioma. PLoS One 2014;9:e91216.
[21] Messiaen LM, Callens T, Mortier G, et al. Exhaustive mutation analysis of the NF1 gene allows identification of 95% of mutations and reveals a high frequency of unusual splicing defects. Hum Mutat 2000; 15:541-55.
[22] Wimmer K, Roca X, Beiglbock H, et al. Extensive in silico analysis of NF1 splicing defects uncovers determinants for splicing outcome upon 5' splice-site disruption. Hum Mutat 2007;28:599-612.
[23] Mautner VF, Kluwe L, Friedrich RE, et al. Clinical characterisation of 29 neurofibromatosis type-1 patients with molecularly ascertained 1.4 Mb type-1 NF1 deletions. J Med Genet 2010;47:623-30.
[24] Wimmer K, Yao S, Claes K, et al. Spectrum of single- and multiexon NF1 copy number changes in a cohort of 1,100 unselected NF1 patients. Genes Chromosomes Cancer 2006;45:265-76.
[25] Kluwe L, Nguyen R, Vogt J, et al. Internal tumor burden in neurofibromatosis Type I patients with large NF1 deletions. Genes Chromosomes Cancer 2012;51:447-51.
[26] Brennan CW, Verhaak RG, McKenna A, et al. The somatic genomic landscape of glioblastoma. Cell 2013;155:462-77.
[27] Reva B, Antipin Y, Sander C. Predicting the functional impact of protein mutations: application to cancer genomics. Nucleic Acids Res 2011;39:e118.
[28] Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012;2:401-4.
[29] Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013;6:pl1.
[30] Odia Y, Orr BA, Bell WR, Eberhart CG, Rodriguez FJ. cMYC expression in infiltrating gliomas: associations with IDH1 mutations, clinicopathologic features and outcome. J Neurooncol 2013;115: 249-59.
[31] Rodriguez FJ, Scheithauer BW, Giannini C, Bryant SC, Jenkins RB. Epithelial and pseudoepithelial differentiation in glioblastoma and gliosarcoma: a comparative morphologic and molecular genetic study. Cancer 2008;113:2779-89.
[32] Kleihues P, Burger PC, Aldape K, et al. Glioblastoma (gliosarcoma). In: Louis DN, Ohgaki H, Wiestler OD, editors. WHO Classification of Tumours of the Central Nervous System. Lyon: IARC; 2007.
[33] Motomura K, Natsume A, Watanabe R, et al. Immunohistochemical analysis-based proteomic subclassification of newly diagnosed glioblastomas. Cancer Sci 2012;103:1871-9.
[34] Carro MS, Lim WK, Alvarez MJ, et al. The transcriptional network for mesenchymal transformation of brain tumours. Nature 2010;463: 318-25.
[35] Kolar K, Freitas-Andrade M, Bechberger JF, et al. Podoplanin: a marker for reactive gliosis in gliomas and brain injury. J Neuropathol Exp Neurol 2014;74:64-74.
[36] Actor B, Cobbers JM, Buschges R, et al. Comprehensive analysis of genomic alterations in gliosarcoma and its two tissue components. Genes Chromosomes Cancer 2002;34:416-27.
[37] Boerman RH, Anderl K, Herath J, et al. The glial and mesenchymal elements of gliosarcomas share similar genetic alterations. J Neuropathol Exp Neurol 1996;55:973-81.
[38] Jiao Y, Killela PJ, Reitman ZJ, et al. Frequent ATRX, CIC, FUBP1 and IDH1 mutations refine the classification of malignant gliomas. Oncotarget 2012;3:709-22.