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Primary intracranial sarcomas with *DICER1* mutation often contain prominent eosinophilic cytoplasmic globules and can occur in the setting of neurofibromatosis type 1

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Compliance with ethical standards

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

Conflict of interest

The authors declare that they have no competing interests related to this study.

Data availability

Scanned image files of the H&E stained slides from which representative images are presented are available for downloading and viewing at the following link: https://figshare.com/projects/Primary_intracranial_sarcoma_with_myogenic_differentiation_and_DICER1_mutation/57704. Sequencing and methylation data files are available from the authors upon request.

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A broad spectrum of primary sarcoma subtypes are now recognized to occur intracranially, presumably arising from mesenchymal progenitor cells within the meningeal covering of the brain and along perivascular Virchow-Robin spaces. Here we report the clinical, radiologic, histologic, and molecular features of three patients with intracranial sarcomas that are unified by the presence of primary intracranial location with meningeal involvement, pleomorphic morphology, high proliferation index, prominent eosinophilic cytoplasmic globules, focal immunophenotypic evidence of myogenic differentiation, and the combination of *DICER1* and *TP53* mutations, along with *ATRX* inactivation and genetic alterations causing activation of the MAP kinase signaling pathway. One patient has neurofibromatosis type 1 (NF1) with multiple cutaneous neurofibromas, cafe-au-lait macules, an optic pathway glioma, and other brain parenchymal lesions characteristic of NF1. This patient was found to have a heterozygous germline nonsense mutation in the *NF1* tumor suppressor gene, along with a second somatic mutation of *NF1* in the primary intracranial sarcoma, indicating that this tumor arose due to biallelic *NF1* gene inactivation. Genome-wide methylation profiling performed on two of the cases revealed that they clustered with the recently described group of tumors termed “primary intracranial spindle cell sarcoma with rhabdomyosarcoma-like features, *DICER1* mutant” [7]. However, while the three tumors in our cohort demonstrate myogenic differentiation as evidenced by focal desmin immunopositivity, none contain identifiable rhabdomyoblasts, they uniformly lack myogenin expression, and are all morphologically best characterized as pleomorphic rather than predominantly spindled. Thus, this patient cohort expands the histologic spectrum and association with familial tumor predisposition syndromes of the primary intracranial sarcomas that cluster with this new methylation subgroup. As such, we suggest a broader designation for this new proposed entity: “Primary intracranial sarcoma, *DICER1*-mutant”.

The one female and two male patients ranged in age at time of initial surgery from 11–20 years (Figure 1a). Patients #1 and #3 had been previously healthy and had no family history of cancer or cutaneous, visceral, or brain lesions other than the primary intracranial sarcomas suggestive of a tumor predisposition syndrome. Patient #2 had a clinical diagnosis of NF1 with multiple cutaneous neurofibromas, café-au-lait macules, an optic pathway glioma, and multifocal T2 signal abnormality within the thalamus, brainstem, and

cerebellum (Supplemental Figure 1 [Online Resource 1]). The patients had initially presented with either seizure or headache. Brain imaging demonstrated complex, solid and cystic, heterogeneously enhancing masses centered within the frontal or parietal lobes with meningeal involvement in all three patients (Figure 1b, Supplemental Figure 1 [Online Resource 1], and Supplemental Table 1 [Online Resource 2]). All tumors demonstrated evidence of prior hemorrhage and were associated with peritumoral edema. Surgical resection was performed for each patient.

Histologic analysis of the three tumors revealed sarcomatous neoplasms that were highly cellular with brisk mitotic activity, foci of necrosis, and intratumoral hemorrhage. Morphology was variable but predominantly consisted of sheets of pleomorphic tumor cells without a discernible growth pattern. A notable histologic feature shared by all three cases was the presence of prominent variablysized brightly eosinophilic cytoplasmic globules (Figure 1c and Supplemental Figures 2-4 [Online Resource 1]). Similar eosinophilic cytoplasmic globules were present at least focally in the majority of the 22 tumors reported by Koelsche et al [7], Case #3 contained a small focus of cartilaginous differentiation. Rhabdomyoblasts with cytoplasmic striations (“strap cells”) were not identifiable in any of the cases, nor was production of osteoid matrix or bone. No associated diffuse glioma was seen in any case to warrant classification as gliosarcoma, although the possibility of a sarcoma-predominant gliosarcoma was considered a diagnostic possibility prior to molecular profiling. Reticulin staining demonstrated abundant intercellular basement membrane deposition in each tumor. Immunohistochemistry demonstrated focal or patchy staining for desmin in all cases, but no myogenin or smooth muscle actin expression was observed (Figure 1d and Supplemental Figures 2-4). Tumor cells were uniformly negative for GFAP, OLIG2, S-100, SOX10, and cytokeratin immunostaining. The Ki67 labeling index was greater than 50% in all three cases. Tumors #1 and #3 demonstrated strong nuclear p53 positivity in the majority of tumor cells, as well as loss of ATRX expression.

In order to assist with diagnostic classification, comprehensive genetic profiling was performed on genomic DNA extracted from formalin-fixed, paraffin-embedded tumor tissue from the three patients, as well as a peripheral blood sample from patient #2, using the UCSF500 Cancer Panel, which assesses approximately 500 cancer-associated genes for mutations, copy number alterations, and structural variants, as well as evaluation of chromosomal copy number changes, microsatellite stability, and somatic mutation burden (Supplementary Table 2 [Online Resource 2] and refs. 3, 4, 5, 8, 9, 10, 11, 12). All three cases demonstrated hotspot missense mutations in exons 24 or 25 of the *DICER1* gene (p.E1705K, p.G1809R, and p.E1813K) located within the Ribonuclease III domain near the C-terminus of the encoded microRNA processing enzyme (Supplemental Table 3 [Online Resource 2]), which have been recurrently seen as confirmed somatic mutations in numerous cystic nephromas, Sertoli-Leydig cell tumors, and other neoplasms known to be driven by *DICER1* mutation (Catalog Of Somatic Mutations In Cancer [COSMIC] database, version 87 release). The *DICER1* mutation in the tumor from patient #1 was homozygous due to copy-neutral loss of heterozygosity of chromosome 14q that eliminated the remaining wildtype allele. In the tumors from patients #2 and #3, there was a second splice site mutation in the *DICER1* gene (c.2436+1G>A and c.5365-1G>A) likely to be present in trans and causing inactivation of the remaining wildtype allele. Such inactivating mutations

affecting one allele together with a hotspot missense mutation affecting the other allele are commonly seen in tumor types known to be driven by *DICER1* mutations [2]. Additionally, all three cases demonstrated inactivating mutations affecting the *TP53* tumor suppressor gene (p.W91*, p.C238F, and p.E258K) accompanied by loss of the remaining wildtype alleles. Two cases demonstrated inactivation of the *ATRX* tumor suppressor gene, one with deep deletion and one with a nonsense mutation (p.W263*). Lastly, each case harbored pathogenic mutations predicted to cause activation of the MAP kinase signaling pathway. The tumor from patient #1 harbored an activating hotspot mutation in the *KRAS* oncogene (p.G12D), and the tumor from patient #3 harbored a hotspot missense mutation in the *PDGFRA* oncogene (p.D842Y) located within the intracellular tyrosine kinase domain that has been recurrently seen as a confirmed somatic mutation in numerous gastrointestinal stromal tumors (COSMIC database, version 87 release). Patient #2 was found to harbor a heterozygous truncating nonsense mutation in *NF1* tumor suppressor gene (p.R192*) in the germline, genetically confirming the clinical diagnosis of NF1. A second somatic missense mutation in the *NF1* gene (p.D2632G) was seen in the tumor, indicating that the sarcoma likely arose, at least in part, due to biallelic *NF1* inactivation. As tumor tissue only without a paired normal sample was sequenced for patients #1 and #3, the somatic versus germline status of the identified *DICER1* and *TP53* mutations could not be determined, but the *DICER1* and *TP53* mutations in patient #2 were both confirmed to be somatic (tumor-specific). All three tumors demonstrated markedly aneuploid genomes with partial gains and losses involving portions of most chromosomes (Supplementary Figure 5 [Online Resource 1]). Genome-wide DNA methylation profiling was performed on cases #1 and #2 as previously described [1, 6] using the Illumina MethylationEPIC BeadChip (850k array) to further characterize these primary intracranial sarcomas with myogenic differentiation and *DICER1* mutation. Unsupervised clustering of DNA methylation patterns demonstrated that both cases clustered with the recently described group of tumors termed “primary intracranial spindle cell sarcoma with rhabdomyosarcoma-like features, *DICER1* mutant” – calibrated score for case #1 was 0.84 and for case #2 was 0.99 (Supplementary Figure 6 [Online Resource 1]).

Following gross total resection, patient #1 was treated with craniospinal radiation and adjuvant chemotherapy with vincristine. He was alive without evidence of disease recurrence at last clinical follow-up approximately six weeks after initial resection. Following gross total resection, patient #2 was treated with cranial radiation and adjuvant chemotherapy with temozolomide. Disease recurrence adjacent to the resection cavity in the left parietal lobe was seen on follow-up imaging at four years after initial resection, and a second resection was recently performed confirming recurrent pleomorphic sarcoma with myogenic differentiation and *DICER1* mutation. Patient #3 was only recently diagnosed and is currently beginning adjuvant therapy.

Overall, this series of three primary intracranial sarcomas has significant similarities with the group of “primary intracranial spindle cell sarcomas with rhabdomyosarcoma-like features, *DICER1* mutant” that were recently reported including primary intracranial location, immunophenotypic evidence of focal myogenic differentiation, overlapping genome-wide methylation profile, co-occurring *DICER1* and *TP53* mutations, and alterations activating the MAP kinase signaling pathway. However, the tumors in our series

have morphology best characterized as pleomorphic rather than spindled, uniformly lack rhabdomyoblasts and myogenin expression, and demonstrate frequent *ATRX* inactivation. As these tumors are all likely to be variants of the same entity we propose the broader nomenclature of “Primary intracranial sarcoma, *DICER1*-mutant”. Though unlikely to be specific, a histologic feature uniformly observed in our case series that should suggest consideration of this tumor entity in an intracranial sarcomatous neoplasm is the presence of prominent eosinophilic cytoplasmic globules. In addition to the previously reported association with the *DICER1* pleuropulmonary blastoma tumor syndrome, we identify that these primary intracranial sarcomas with *DICER1* mutation can also occur in the setting of NF1, thereby extending the tumor spectrum associated with this common familial tumor syndrome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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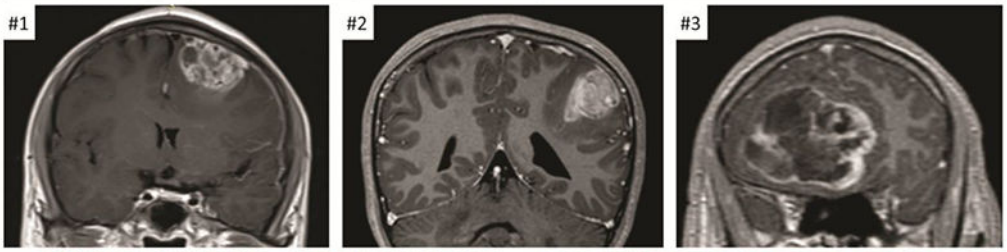
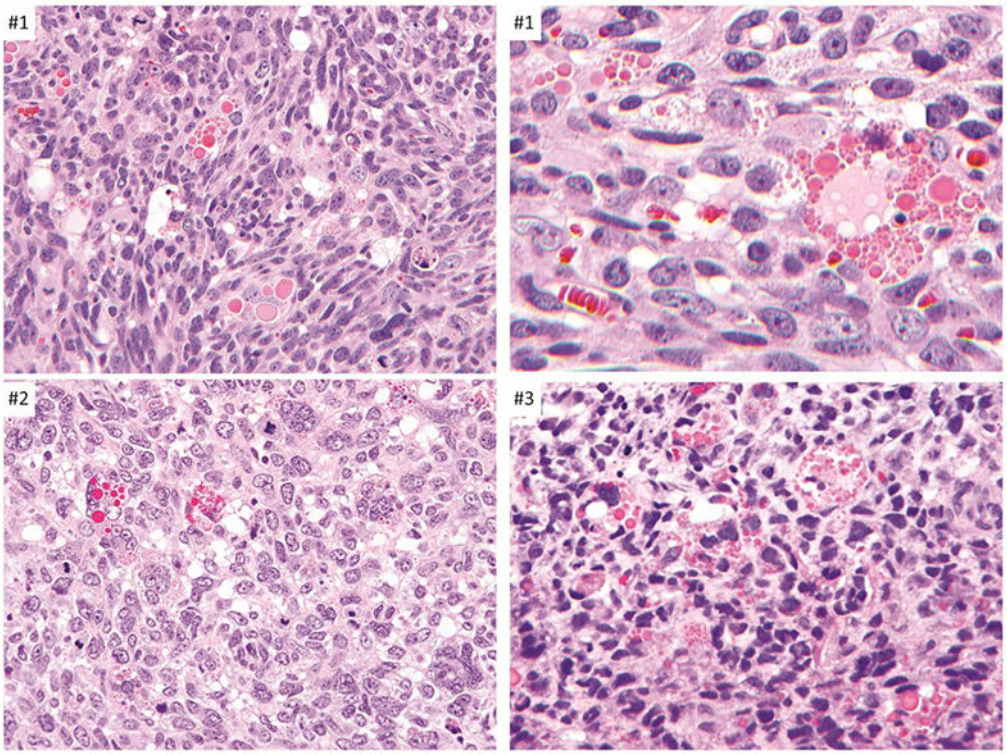
a

Patient	Age (yrs)	Sex	Tumor location	Presenting symptoms	Pathogenic alterations
#1	20	M	Brain, left frontal	seizure	DICER1 p.E1813K, TP53 p.C238F, KRAS p.G12D, ATRX deep deletion
#2	11	F	Brain, left parietal	headaches	DICER1 p.G1809R, DICER1 c.2436+1G>A, TP53 p.W91*, NF1 p.D2632G, NF1 p.R192*(germline)
#3	19	M	Brain, right frontal	seizure	DICER1 p.E1705K, DICER1 c.5365-1G>A, TP53 p.E258K, PDGFRA p.D842Y, ATRX p.W263*, PTEN p.K125N

b



c



d

Patient	Reticulin	SMA	Desmin	Myogenin	S-100	GFAP	OLIG2	Keratin	ATRX	p53
#1	+++	neg	patchy +	neg	neg	neg	neg	neg	loss	+++
#2	+++	neg	patchy +	neg	neg	neg	neg	neg	N/A	N/A
#3	+++	neg	focal +	neg	neg	neg	neg	neg	loss	+++

Figure 1.

Clinical, radiologic, histologic, and molecular features of three patients with primary intracranial pleomorphic sarcomas with myogenic differentiation and *DICER1* mutation. **a**, Clinical features and pathogenic molecular alterations identified. **b**, Pre-operative T1-weighted post-contrast MR images. **c**, Representative images from H&E stained sections showing pleomorphic sarcomas with brisk mitotic activity and prominent eosinophilic cytoplasmic globules. **d**, Summary of reticulin staining and immunohistochemical results.