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SHORT COMMUNICATION

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Evidence of neural crest cell origin of a DICER1 mutant CNS sarcoma in a child with DICER1 syndrome and NRAS-mutant neurocutaneous melanosis

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DICER1 mutant CNS sarcoma is a newly defined entity that may present with various morphologies and is characterised by a distinct DNA methylation profile [1–3]. Tumours occur sporadically or in the context of DICER1 syndrome as well as rarely in neurofibromatosis type 1 [1, 4]. Most tumours present with additional mutations in *TP53* and RAS-pathway genes [1, 4–6]. Although the mutational landscape of DICER1 mutant CNS sarcomas has been investigated, not much is known about the cellular origin of this entity. We present a male

neonate with severe muscular hypotonia and multiple melanocytic naevi. The prenatal period was uneventful except for a large arachnoid cyst with cerebellar hypoplasia. At the age of 6 weeks, he developed paraplegia of the lower limbs. A magnetic resonance imaging showed brain melanosis (Figure 1A) and a thoracic intraspinal and paraspinal tumour (Figure S1, online resource). The tumour extended through the intravertebral foramen and was focally abutting the pleura (see Figure S1c), but did not extend into the pleural cavity. Intrapulmonary or distant lesions were not identified.

The tumour was incompletely resected and demonstrated variable degree of maturation. Low-grade mesenchymal areas with scattered lipomatous cell nests and numerous pseudo-meissnerian corpuscles (PMCs) predominated over undifferentiated and sarcomatous, MPNST-like areas (approximately 70% vs 30% of tumour tissue; Figures 1A and S2, online resource). The immunohistochemical expression pattern was heterogeneous: PMCs strongly expressed WT1, S100, SOX10 and CD56. The sarcomatous component demonstrated focal reactivity for SOX10 as well as several dispersed WT1 and p75 positive and single S100 and MiTF positive tumour cells. CD56 was strongly expressed by the sarcomatous component, but negative in undifferentiated tumour areas. Nuclear p53 accumulation was mainly seen in the sarcomatous and primitive areas, but was absent in low-grade areas. The tumour cells did not express desmin, SMA, MyoD1, TLE-1, MAP2, synaptophysin or chromogranin A. The proliferation activity was very high in undifferentiated areas (Ki67 90%), markedly increased in sarcomatous areas (15–25%) and low in mesenchymal areas with PMCs (2%) (Figure S2, online resource).

Primitive and sarcomatous areas of the tumour were both classified as primary intracranial sarcoma, DICER1-mutant based on DNA methylation profiling (brain tumour classifier v12.5: classifier score 0.99; www.molecularneuropathology.org, [7]). The sarcomatous tumour component showed a flat copy number profile, whereas the primitive tumour component demonstrated partial gain of chromosome 9 and partial loss of chromosome 11 (Figure S3, online resource). Next-generation sequencing (NGS) demonstrated a constitutional pathogenic variant in *DICER1* c.3007C > T p.(Arg1003*) (RefSeq Transcript NM_030621.4) inherited from the father with biallelic inactivation of *DICER1* by a second pathogenic *DICER1* c.5439G > T p.(Glu1813Asp) hotspot mutation being confined to the malignant tumour areas (Table 1). A clonal *NRAS* c.181C > A p.Gln61Lys variant (RefSeq Transcript NM_002524.5) was detected in low- and high-grade tumour areas as well as in two biopsies of skin affected by melanocytic nevi resulting in the diagnosis of neurocutaneous melanosis caused by postzygotic mosaicism of *NRAS*.

The patient was treated according to the EU-RHAB protocol and response to therapy improved after MEK-inhibition was added to the chemotherapy regimen. He finally underwent surgery to remove the residual paraspinal tumour components 20 months after initial diagnosis. The residual tumour consisted of low-grade fibrolipomatous tissue with an increased number of PMCs compared to the initially resected lesion. Malignant tumour areas were not observed. In one area, a smooth transition into differentiating neuronal tissue with a neuropil-like matrix and dysplastic neurons was demonstrated. The neuronal tumour component stained positive for CD56, S100, synaptophysin, and MAP 2 (Figure S4, online resource). The proliferation and mitotic activity was low. The methylation profile was not classifiable (brain tumour v12.5 and sarcoma v12.2 classifier scores < 0.9). NGS of the residual tumour tissue revealed both constitutional *DICER1* and *NRAS* pathogenic variants, whereas the somatic *DICER1* mutation (p.(Glu1813Asp)) was only detected at a very low allele frequency (2.8%) in the neuronal areas (Table 1).

A final descriptive diagnosis of a "heterogeneous, neural lesion with sarcomatous, MPNST-like tumour component (with the methylation profile of a DICER1 mutant sarcoma) and a maturing neuronal component in the context of DICER1 syndrome and neurocutaneous melanosis" was made to reflect on the complexity and heterogeneity of the lesion. The child is in complete remission two and a half years after diagnosis and discontinuation of MEK inhibition.

DICER1–associated sarcomas may arise in various anatomical locations and share similar morphological features [8]. Tumours are usually described as malignant mesenchymal neoplasms with frequent rhabdomyoblastic differentiation, but cases with other morphologies ranging from primitive PNET-like to MPNST-like appearances as well as cartilaginous differentiation and osteoid formation have been reported [1, 2, 4]. McCluggage et al. [8, 9] suggested that these tumours are part of a common tumour spectrum and proposed a unifying nomenclature for DICER1 mutant sarcomas. The hypothesis is further supported by preliminary evidence of a common epigenetic profile of DICER1 mutant CNS sarcomas and two DICER1 mutant embryonal rhabdomyosarcomas of the uterus shown by Kölsche et al. [1].

The expanding histological spectrum of DICER1 mutant sarcomas argues for a precursor cell capable of multilineage differentiation regardless of the site. Neural crest cells migrate during early embryogenesis throughout the body and have a broad differentiation capacity ranging from neural tissue (melanocytes, neurons, Schwann cells) to mesenchymal tissue (chondrocytes, fibroblasts, and adipocytes; Figure 1A) [10]. In the case presented here, the patient developed a

Key Points

- DICER1 mutant CNS sarcomas can arise in patients with neurocutaneous melanosis and DICER1 syndrome.
- These tumours may present with neural differentiation and a high degree of morphological and genetic intratumoural heterogeneity.
- Pleuropulmonary blastoma represents a relevant differential diagnosis in children with DICER1 mutant CNS sarcoma in a spinal location.
- MEK inhibition in addition to chemotherapy in a child with DICER1 mutant sarcoma resulted in improved response and complete remission.

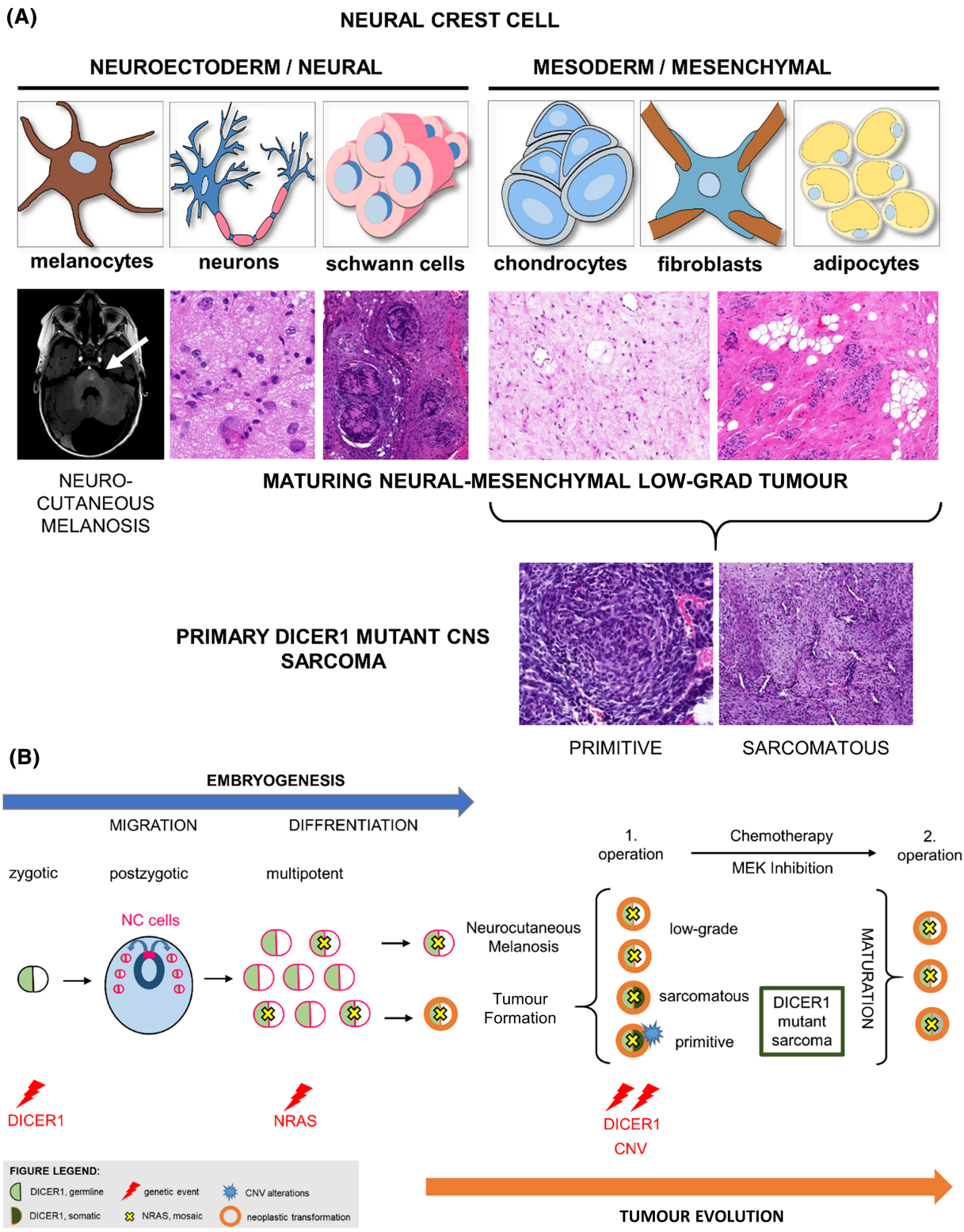

FIGURE 1 Characteristics of a spinal tumour in a child with DICER1 syndrome and neurocutaneous melanosis. (A) Cerebellar hypoplasia and large arachnoid cyst and T1 hyperintense melanin deposits in pons and medulla oblongata (→). Formation of a tumour demonstrating various lineage differentiation (e.g., dysplastic neurons and neuropil, pseudo-meissnerian corpuscles – specialised schwannian cells, fibrovascular to immature chondroid matrix, adipocyte cell nests) with a malignant tumour component arising from the mesenchymal areas (primitive and sarcomatous differentiation; see Figure S2, online resource). (B) Synopsis: A multistep process of accumulating genetic events during embryogenesis in neural crest cell precursor cells leads to tumour formation and transformation in a DICER1 mutant sarcoma. Genetic intratumoural heterogeneity influences the degree of maturation and malignancy. Abbreviations: CNV, copy number alterations; NC, neural crest

TABLE 1 Summary of genetic results of different specimens and tumour areas

Specimen	Tumour component	DICER1 (MAF)	DICER1 (MAF)	NRAS (MAF)
Blood		-	c.3007C > T p.(Arg1003*) (heterozygous)	-
Skin right arm		NA	NA	c.181C > A p.Gln61Lys (20%)
Skin left leg		NA	NA	c.181C > A p.Gln61Lys (7%)
Initial tumour	Malignant	p.(Glu1813Asp) (36.4%)	c.3007C > T p.(Arg1003*) (40.3%)	c.181C > A p.Gln61Lys (80.4%)
	Low-grade	-	c.3007C > T p.(Arg1003*) (43.7%)	c.181C > A p.Gln61Lys (84.1%)
Residual tumour	Matured neural-mesenchymal	p.(Glu1813Asp) (2.8%)	c.3007C > T p.(Arg1003*) (44%)	c.181C > A p.Gln61Lys (31.3%)

Note: Next-generation sequencing (customised Nextera-DNA-Flex for Enrichment protocol, MiSeq Illumina) results from blood, two skin biopsies, malignant and low-grade component of the initial tumour and the residual tumour. The malignant tumour areas demonstrated biallelic inactivation of *DICER1*, whereas in other tissues, only the germline mutation was detected. The mosaic *NRAS* mutation was absent in the blood sample, but present in affected skin and both components of the tumour (MAFs in tumour samples may indicate a copy number neutral loss of heterozygosity of chromosome 1p, which cannot be detected by CNV analysis from methylation data). Variant annotation according to HGVS-nomenclature (Human Genome Variation Society), Transcripts *DICER1* (NM_030621.4) and *NRAS* (NM_002524.5).
Abbreviations: MAF, mutant allele frequency; NA, not available.

highly heterogeneously differentiated neural-mesenchymal tumour with both constitutional *DICER1* and mosaic *NRAS* mutation. In patients with neurocutaneous melanosis, the postzygotic *NRAS* mutation only affects a subpopulation of neural crest cells, but cannot be detected in the germline of the patient. We therefore deduce that the DICER1 mutant sarcoma with *NRAS* mutation in our patient arose from a neural crest cell by acquisition of a second mutation in *DICER1* and chromosomal alterations (Figure 1B). As the tumour was epigenetically classified as primary intracranial sarcoma, DICER1-mutant and the methylation profile of a cancer cell strongly reflects the cell of origin [7], it is tempting to speculate that DICER1 mutant sarcomas in general are derived of neural crest cells which needs to be demonstrated in further studies. A synergistic effect in tumour formation of the germline *DICER1* mutation and the somatic *NRAS* mutation may be assumed as *NRAS* mutations are found in approximately 10% of DICER1 mutant CNS sarcomas [1, 2].

It has been hypothesized that *NRAS* mutations in neurocutaneous melanosis occur before the differentiation of neural crest cells into the melanocytic lineage [11, 12]. A variety of non-melanotic tumours have been reported to arise in neurocutaneous melanosis patients, among them rhabdomyosarcomas and malignant peripheral nerve sheath tumours [12–14]. The level of differentiation and the environment of migrating neural crest cells at the time-point of acquisition of *NRAS* and/or *DICER1* mutations during embryogenesis potentially influence the resulting phenotype and the variability in histological appearances of these tumours.

Here, we showed that DICER1 mutant CNS sarcoma may arise in spinal location and in the context of two co-occurring cancer predisposing conditions. Our temporal and spatially distinct analysis argues not only for a high morphological, but also genetic heterogeneity within DICER1 mutant CNS sarcomas, possibly relevant for future treatment strategies. Our study also provides a rationale to investigate DICER1 deficiency in neural crest cells in cell culture and mice.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ETHICS STATEMENT

Written permission for publication was obtained from the parents of the child.

AUTHOR CONTRIBUTIONS

LS, WH and AK interpreted histopathological, immunohistochemical and molecular findings. DH, SH, KWP, IW and LS performed next-generation sequencing and assisted with sequencing interpretation. MN and AT interpreted radiological images. UT and VP provided neurosurgical clinical care. RR, PH, KH and BZ provided neuro-oncology clinical care and assisted in data collection. LS prepared the figures and wrote the manuscript. All authors edited the manuscript and approved the final version.

DATA AVAILABILITY STATEMENT

Data are available upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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