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CORRESPONDENCE

SMARCA4-mutated atypical teratoid/rhabdoid tumors are associated with inherited germline alterations and poor prognosis

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Atypical teratoid/rhabdoid tumors (AT/RT) are highly malignant rhabdoid brain tumors predominantly affecting young children. Biallelic inactivation of the *SMARCB1* gene (also known as hSNF5/INI1) is the characteristic underlying genetic lesion [6]. SMARCB1 is a core member of the chromatin-remodeling complex, playing a key role in the regulation of proliferation and differentiation [16]. Germline alterations of *SMARCB1* predisposing to the development of malignant rhabdoid tumors [Rhabdoid Tumor Predisposition Syndrome-1 (OMIM#01607)] are encountered in about one-third of children with AT/RT; the majority of *SMARCB1* germline mutations occur de novo [3]. Some children, however, develop AT/RT without loss of SMARCB1 protein expression [4]. We have recently demonstrated biallelic inactivation of *SMARCA4* (encoding the SMARCA4 protein also named BRG1), one of the mutually exclusive ATPase subunits of the SWI/SNF chromatin-remodeling complex, as well as transmission of *SMARCA4* germline mutations in two families [14, 18]. Here, we show that *SMARCA4*-mutated AT/RT are associated with a higher frequency of inherited germline alterations and worse prognosis as compared to SMARCB1-deficient AT/RT.

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Clinical information as well as neuropathological and molecular genetic findings on nine children harboring AT/RT with retained SMARCB1 protein expression was retrieved, including the above-mentioned cases [5, 14, 18]. All tumors affected young children [median age 9 months (range 0–22 months)]. On neuropathological examination, the tumors displayed rhabdoid tumor cells and the characteristic immunohistochemical staining profile of AT/RT with the notable exception of retained (normal) SMARCB1 protein expression. As shown in Table 1 and illustrated in Supplemental Material 1, *SMARCA4* mutations resulting in loss of SMARCA4 protein expression were identified in eight tumors. One additional tumor showed retained (normal) SMARCA4 protein expression. Sequencing of *SMARCA4* and FISH analysis were carried out as described previously [14] and revealed nonsense *SMARCA4* mutations in six cases, while two cases showed wild-type *SMARCA4* on sequencing but a chromosomal deletion or findings suggestive of breakage affecting the *SMARCA4* region, respectively, on FISH analysis. Furthermore, in the tumor showing retained SMARCA4 protein expression, a missense mutation (c.2335G>A) predicting an amino acid exchange (p.Asp779Asn) in the ATP-binding region of the ATPase domain was identified. Remarkably, in this case as well as in five of the other cases, the combined sequencing and FISH data suggest copy-neutral loss of heterozygosity (LOH) as mechanism of somatic inactivation of the second allele. Since *SMARCA4* missense mutations have not yet been associated with AT/RT, we analyzed the possible impact of the p.Asp779Asn mutation on *SMARCA4* function with respect to its ATPase activity by homology modeling and superimposition on additional structures with bound ATP or DNA/RNA (Supplemental Material 2). p.Asp779 is a highly conserved amino acid and part of the Walker A motif ATP-binding site. Its mutation to asparagine is expected to affect ATP binding and hence the ATPase-dependent chromatin remodeling activity of the SWI/SNF complex.

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SMARCA4 germline mutations and inheritance from non-affected parents (suggesting incomplete penetrance) were demonstrated in 6/7 children that could be examined (Table 1). As compared to 33 SMARCB1-deficient AT/RT from the European Rhabdoid Tumor Registry (EURHAB), for which information on germline mutation status and follow-up was available, the proportion of germline alterations was higher in *SMARCA4*-mutated AT/RT (6/7 vs. 9/33, Fisher's exact test $P < 0.01$). As shown in Fig. 1, overall survival estimates were shorter in *SMARCA4*-mutated AT/RT as compared to *SMARCB1* mutated AT/RT [3 months (95 % confidence intervals 0–6 months) vs. 24 months (95 % confidence intervals 17–31 months), Log-Rank test $P < 0.001$]. Multivariate analysis (Cox regression analysis using the Wald approach) confirmed this effect to be independent of young age (<1 year) and germline mutation status ($P < 0.01$).

Throughout the last decade, immunohistochemistry for SMARCB1 protein expression has contributed to an earlier diagnosis of SMARCB1-deficient AT/RT [9]. Our findings strongly argue for the investigation of SMARCA4 expression status in all tumors suspicious of AT/RT, but showing retained SMARCB1 staining. The frequent presence of *SMARCA4* germline alterations and their parental transmission should prompt genetic counseling of affected families.

The fact that the child harboring an AT/RT with a homozygous missense mutation showed relatively long survival (28 months) as compared to tumors with loss of SMARCA4 protein could well point toward some residual function of the mutated protein. Mutations of *SMARCA4* have been identified in Coffin–Siris syndrome, a developmental disorder with intellectual disabilities [15]. In contrast to most *SMARCA4* mutations associated with cancers, the mutations causing Coffin–Siris syndrome (missense mutations and in frame deletions) did not abolish SMARCA4 protein expression. Interestingly, *SMARCA4* missense mutations have also been reported in medulloblastoma [8, 11, 13]. Even though most of these mutations were heterozygous, they might play a role in tumor biology, e.g., by increasing anaphase bridge formation [2]. Recently, germline and somatic mutations of the *SMARCA4* gene resulting in loss of SMARCA4 protein expression have been linked to small cell carcinoma of the ovary hypercalcemic type (SCCOHT), a highly aggressive tumor affecting young women [7, 12, 17]. It will be interesting to investigate genetic and epigenetic similarities and differences of SMARCA4-deficient AT/RT and SCCOHT. Furthermore, it remains to be determined which factors contribute to the incomplete penetrance observed in families with *SMARCA4* germline mutations.

When interpreting our findings, the limitations related to a retrospective study must be taken into account. In children harboring SMARCB1-deficient AT/RT, germline

Table 1 Patient characteristics and molecular genetic findings

Patient	Age (months)	Sex	Location	SMARCB1 protein expression	SMARCA4 protein expression	*SMARCA4* somatic mutation status		*SMARCA4* germline status	Survival (months)	Comments
						Sequencing	FISH			
#1	10	Female	Infratentorial	Retained (normal)[a]	Lost	c.3565C>T (p.Arg1189*), loss of heterozygosity (LOH)	No alterations nuc ish 19p13 (RP11-91O21 × 2, RP11-90A8 × 2, RP11-19I2 × 2)	c.3565C>T (p.Arg1189*)	2	Patient III2 reported in [14]. Mutation also present in the germline of the healthy father, sister died from a malignant rhabdoid tumor of the kidney
#2	9	Male	Supratentorial	Retained (normal)[a]	Lost	c.2032C>T (p.Gln678*) LOH	No alterations nuc ish 19p13 (RP11-360D23 × 2, RP11-90A8 × 2, CTD-3184H13 × 2, RP11-19I2 × 2)	Not available	6	Family declined further genetic testing. Reported in [5]
#3	0	Female	Infratentorial	Retained (normal)	Lost	Possibly compound heterozygous c.3229C>T (p.Arg1077*) and c.3533G>A (p.Trp1178*)	Not analyzed	c.3533G>A (p.Trp1178*)	1	Mutation also present in the germline of the mother, in which a malignant tumor of the ovary with biallelic inactivation of *SMARCA4* was diagnosed. Reported in [18]
#4	9	Female	Infratentorial	Retained (normal)	Lost	c.4039_4076del38 p.Ile1347*, LOH	No alterations nuc ish 19p13 (RP11-360D23 × 2, CTD-318H13 × 2)	c.4039_4076del38 p.Ile1347*	1	Mutation also present in the germline of three healthy relatives (father, paternal aunt and the paternal grandmother)
#5	22	Male	Supratentorial	Retained (normal)	Lost	Wild type	Deletion of one allele and break of the other nuc ish 19p13 (RP11-360D23 × 1, CTD-3184H13 × 1) (RP11-360D23 sep CTD-318H13 × 1)	No alterations on FISH and sequencing	3	
#6	3	Male	Infratentorial	Retained (normal)	Lost	Wild type	Unbalanced break, likely deletion from polyploid/polysomic clone nuc ish 19p13 (CTD-3184H13 × 2–3, RP11-360D23 × 4–8)	Not available	4	
#7	1	Male	Supratentorial	Retained (normal)[a]	Lost	c.2491_2505del15 p.Lys831_Lys835del LOH	No alterations nuc ish 19p13 (RP11-360D23 × 2, CTD-318H13 × 2)	c.2491_2505del15 p.Lys831_Lys835del	10	
#8	3	Female	Supratentorial	Retained (normal)	Lost	c.3407delG p.Gly1136Alafs*4, LOH	No alterations nuc ish 19p13 (RP11-360D23 × 2, CTD-318H13 × 2)	c.3407delG p.Gly1136Alafs*4	1	Mutation also present in the germline of the healthy father
#9	17	Male	Infratentorial	Retained (normal)[a]	Retained (normal)	c.2335G>A (p.Asp779Asn), LOH	No imbalances (10 % of cells with break within *SMARCA4*) nuc ish 19p13 (RP11-360D23 × 2, CTD-318H13 × 2)	c.2335G>A (p.Asp779Asn)	28	Mutation also present in the germline of the healthy father

[a] As well as lack of underlying genetic alterations of the *SMARCB1* locus demonstrated on FISH and sequencing

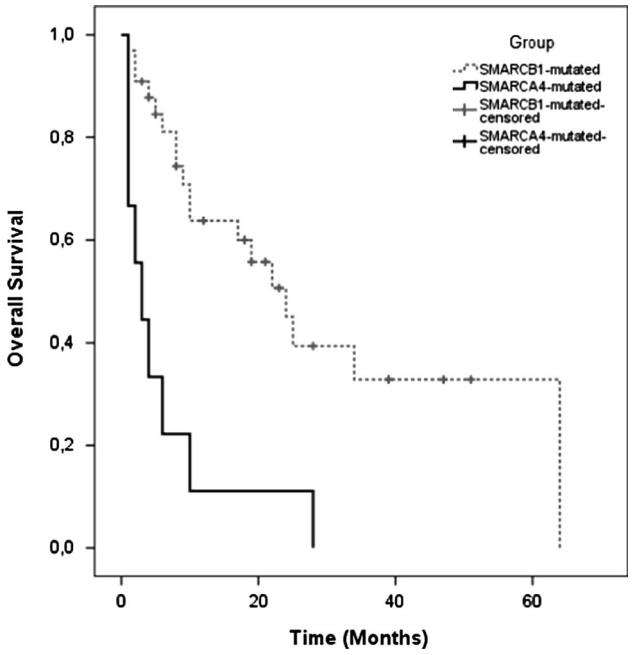

Fig. 1 Survival analysis. Overall survival of children harboring *SMARCA4*-mutated AT/RT ($N = 9$, *solid line*) as compared to *SMARCB1* mutated tumors (*dotted line*, $N = 33$). Log-Rank test $P < 0.001$

alterations are usually only examined in the context of very young age and biologically aggressive tumors, which are also reflected by the relatively short survival of the 33 SMARCB1-deficient AT/RT with known germline mutation status that were used for comparison. However, despite such potential bias, survival of children harboring *SMARCA4*-mutated AT/RT was even shorter. Of note, this effect was also independent from factors commonly associated with detrimental prognosis, namely young age and germline mutation status [1, 10].

In conclusion, genetic alterations of *SMARCA4* in AT/RT are associated with a high rate of inherited germline mutations and aggressive biological behavior. These results should prompt screening for *SMARCA4* alterations in infants with malignant brain tumors.

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