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CORRESPONDENCE

Poorly differentiated chordoma with SMARCB1/INI1 loss: a distinct molecular entity with dismal prognosis

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Chordomas are tumors of the skull base and spine thought to arise from remnants of the notochord. Expression of cytokeratins and S100 is frequent and nuclear expression of brachyury, a transcription factor important for axial development, has been shown to be a sensitive and fairly specific diagnostic marker [6]. For pediatric chordomas showing cytological atypia, increased mitotic activity, increased cellularity and an unstructured growth pattern, the term "poorly differentiated chordoma" has been coined [2, 7]. Interestingly, poorly differentiated chordomas are not only associated with an aggressive clinical course and high mortality [2], but also with loss of SMARCB1 expression [7, 10]. Loss of SMARCB1 (also known as hSNF5/INI1), a core member of the SWI/SNF chromatin remodeling complex, is the hallmark of atypical teratoid/rhabdoid

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tumor (AT/RT), a brain tumor in young children also demonstrating a highly aggressive biological behavior. Since AT/RT invading the skull and even clival location are on record [4, 9], it remains uncertain if poorly differentiated chordoma represents a distinct entity or part of the AT/RT spectrum instead. Here we show that poorly differentiated chordoma can be clearly distinguished not only from AT/RT, but also from conventional chordoma by DNA methylation profiling.

Formalin-fixed paraffin embedded samples of seven poorly differentiated chordomas (including four previously reported cases [1, 7]) were retrieved from our archives. In addition to the primary tumors, in one case material from a recurrence could be examined. Data on treatment and overall survival were compiled by reviewing patient records. For comparison, samples of 14 conventional chordomas as well as ten AT/RT of each of three recently described molecular subgroups (i.e. TYR, MYC and SHH) [3] were evaluated. Protein expression of brachyury and SMARCB1 was examined using immunohistochemistry. Fluorescence in situ hybridization (FISH) of the *SMARCB1* locus, *SMARCB1* sequencing and multiplex ligation-dependent probe amplification (MLPA) using the SALSA MLPA P258 (SMARCB1) kit (MRC-Holland) were performed in tumors showing SMARCB1 loss. All samples were analyzed using the Illumina Infinium Human Methylation 450k Bead Chip (see supplemental methods).

The median age of the four boys and three girls harboring poorly differentiated chordomas was 7 years (range 1–11 years; for details see supplementary Table 2). The median overall survival was only 9 months (95 % confidence interval of 6–12 months). On histopathological examination, all poorly differentiated chordomas and conventional chordomas exhibited nuclear brachyury expression, and nuclear brachyury expression was also observed in 2/30 AT/RT (Fig. 1b). In contrast, all poorly differentiated chordomas and AT/RT showed loss of SMARCB1 expression, while SMARCB1 expression was retained in conventional chordomas. Unsupervised clustering analysis of the 52 methylation profiles using the 5000 most

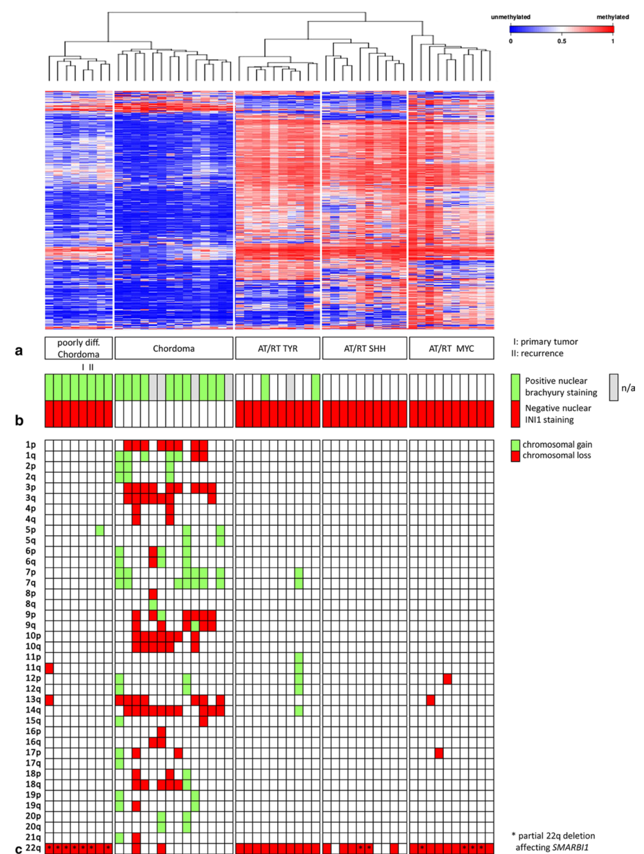

Fig. 1 Molecular profiling of poorly differentiated chordoma versus conventional chordoma and AT/RT. Unsupervised clustering of methylation profiles of 52 samples using the 5000 most variable probes (**a**), immunohistochemistry for brachyury and SMARCB1 (**b**) as well as copy number alterations derived from 450k data (**c**) of seven poorly differentiated chordomas and one recurrence (I: primary tumor, II: recurrence), 14 conventional chordomas and AT/RT of the molecular subgroups TYR, SHH and MYC ($n = 10$ each). *N/A* no material available for staining

differentially methylated CpG sites across all samples identified five distinct methylation groups, including two distinct chordoma clusters, representing the poorly differentiated chordomas and the conventional chordomas, respectively, both clustering apart from the three molecular AT/RT subgroups (Fig. 1a). Copy number profiles were derived from intensity measures of the methylation probes and indicated 22q losses affecting the *SMARCB1* region in all poorly differentiated chordomas and the vast majority of AT/RT as the only recurrent alteration (Fig. 1c and Supplementary Fig. 1), whereas conventional chordomas showed complex alterations including frequent losses of chromosomes 14q (10/14; 71 %), 3p (9/14; 64 %), 1p (8/14; 57 %) and 13q (7/14; 50 %) as well as chromosome 7q gains (7/14; 50 %). Heterozygous or homozygous deletions affecting the *SMARCB1* region were confirmed on FISH and/or MLPA (4/7 and 2/7 poorly differentiated

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chordomas, respectively), while *SMARCB1* point mutations were absent on sequencing (0/7 cases).

Our data confirm the detrimental prognosis of poorly differentiated chordomas and clearly demonstrate that poorly differentiated chordoma can be reliably separated from conventional chordoma and AT/RT by a distinct methylation profile, which may be diagnostically helpful in difficult cases. Whereas conventional chordomas showed complex copy number alterations as described previously [5], poorly differentiated chordomas mainly displayed isolated losses affecting the *SMARCB1* region in 22q11 as encountered in AT/RT. The finding that *SMARCB1* was inactivated in poorly differentiated chordomas by deletions rather than point mutations is in line with previous observations [10] and has been also described in other non-rhabdoid SMARCB1-deficient soft tissue tumors such as epithelioid sarcoma [8]. It remains to be determined, how different types of *SMARCB1* mutations result in a heterogeneous group of tumors, which may well involve different cells of origin, different developmental stages, and a diverse genetic or epigenetic background in which these mutations occur. Given the relatively low number of cases examined, further prospective molecular studies would be highly desirable.

In conclusion, poorly differentiated SMARCB1-negative chordoma represents a molecularly distinct entity with dismal prognosis.

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