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CORRESPONDENCE

CDKN2A deletion in supratentorial ependymoma with *RELA* alteration indicates a dismal prognosis: a retrospective analysis of the HIT ependymoma trial cohort

Stephanie T. Jünger[1,8] · Felipe Andreiuolo[1] · Martin Mynarek[2] · Inken Wohlers[3,9] · Sven Rahmann[3] · Ludger Klein-Hitpass[4] · Evelyn Dörner[1] · Anja zur Mühlen[1] · Natalia Velez-Char[1] · Katja von Hoff[2] · Monika Warmuth-Metz[5] · Rolf-Dieter Kortmann[6] · Beate Timmermann[7] · Andre von Bueren[2,10] · Stefan Rutkowski[2] · Torsten Pietsch[1]

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Pediatric supratentorial ependymomas with *RELA* fusions (RELA-EP) have been identified as a unique novel tumor entity [9, 10]. Fusions between *C11orf95* and *RELA* pathologically activate the NFκB signaling pathway indicated by nuclear accumulation of p65-RelA. Deletions of *CDKN2A* encoding the negative cell-cycle regulator p16 have been described in a subset of supratentorial ependymomas, associated with worse outcome [2, 5, 7]. We assessed the frequency and prognostic impact of *CDKN2A* deletions in a cohort of 54 RELA-EP in children treated according to HIT2000-E protocols (for detailed demographic information, see supplementary materials and methods and supplementary table 1).

High-resolution, genome-wide copy number profiles were generated by molecular inversion probe (MIP) assay. Chromosomal breaks were identified within the *C11orf95* and *RELA* genes corresponding to fusion transcripts (Fig. 1a, d). All cases showed pathological nuclear accumulation of p65-RelA as a hallmark of RELA-EP (Fig. 1f). Homozygous deletion (complete loss) of *CDKN2A* was detected in 9 of 54 (16.7%) cases (Fig. 1c); and 8 of these (88.9%) showed a concordant complete loss of p16 protein (Fig. 1g). In one case, few tumor cells expressed p16 protein indicating retained *CDKN2A* alleles in single cells. Fourteen cases (25.9%) harbored a hemizygous deletion of *CDKN2A*. In these, p16 protein was retained in 92.9% of cases tested—one case lacked p16 protein expression indicating the inactivation of the second allele by alternative mechanisms. Thirty-one of 54 cases (57.4%) had no deletion of *CDKN2A;* all showed p16 protein expression (Fig. 1h). Immunohistochemistry for p16, therefore, may serve as a surrogate for complete *CDKN2A* loss, but cannot differentiate between hemizygous and wild-type status. There was no statistical association between *CDKN2A* deletions and mitotic activity as previously described in *IDH*-mutant glioma [1]. The

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✉ Torsten Pietsch
t.pietsch@uni-bonn.de

1 Department of Neuropathology, Institute of Neuropathology, DGNN Brain Tumor Reference Center, University of Bonn, Venusberg-Campus 1, 53127 Bonn, Germany

2 Department of Pediatric Hematology and Oncology, University Hospital Hamburg-Eppendorf, Hamburg, Germany

3 Genome Informatics, Institute of Human Genetics, University of Duisburg-Essen, Essen, Germany

4 Department of Cell Biology (Tumor Research), University of Duisburg-Essen, Essen, Germany

5 Institute of Diagnostic and Interventional Neuroradiology, University Hospital Würzburg, Würzburg, Germany

6 Department of Radiation Oncology, University Hospital Leipzig, Leipzig, Germany

7 Westdeutsches Protonentherapiezentrum, Essen, Germany

8 Present Address: Department of Neurosurgery, University of Cologne Medical Center, Cologne, Germany

9 Present Address: Medical Systems Biology Division, Lübeck Institute of Experimental Dermatology and Institute for Cardiogenetics, University of Lübeck, Lübeck, Germany

10 Present Address: Division of Pediatric Hematology and Oncology, Department of Pediatrics, Obstetrics and Gynecology, University Hospital of Geneva, Geneva, Switzerland

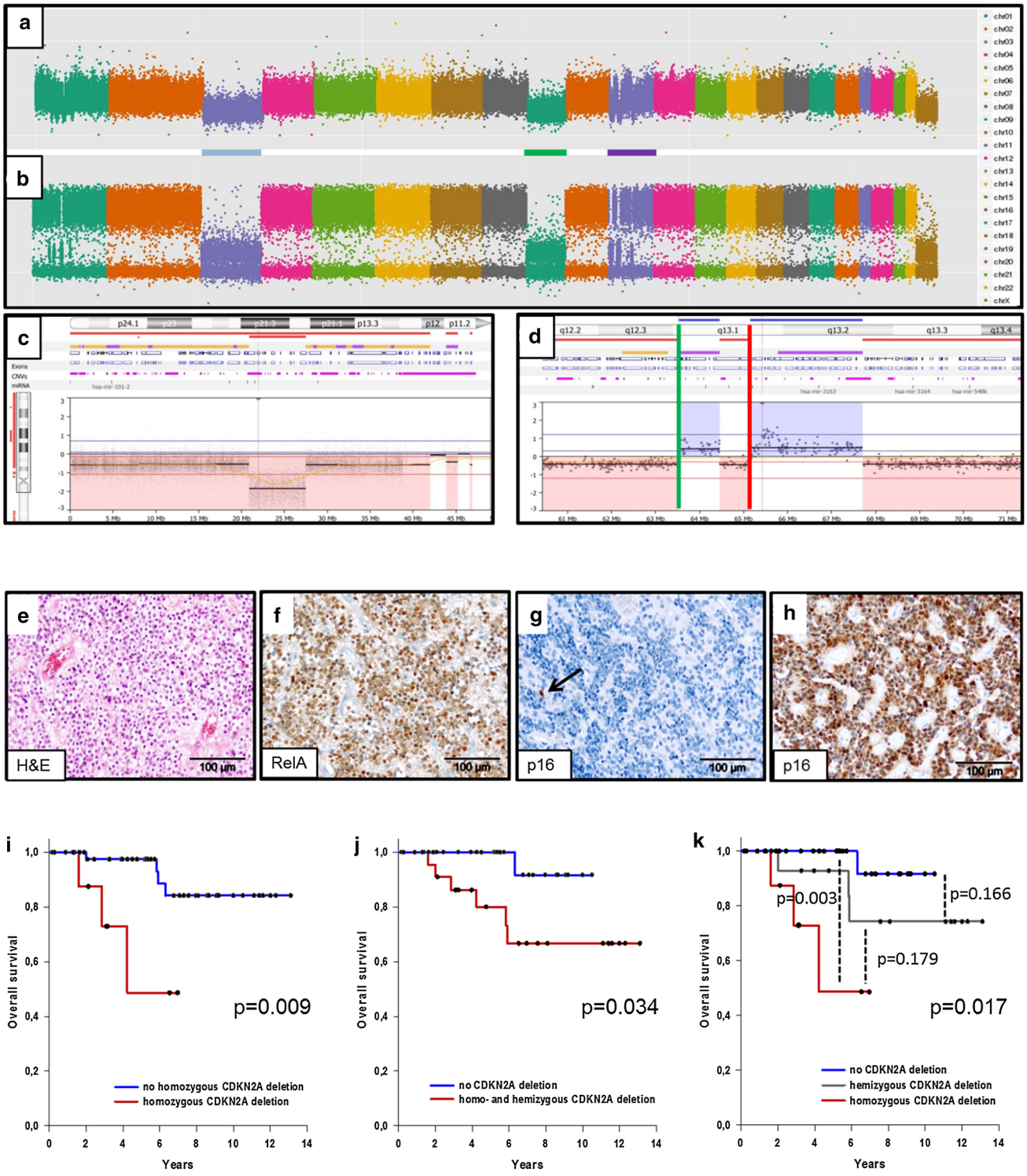

Fig. 1 **a** Genomic copy number profile and **b** allele distribution (MIP) of a RELA-EP showing chromothripsis of chromosome 11; **c** case with homozygous *CDKN2A* deletion; **d** case showing breaks in *C11orf95* (green bar) and *RELA* (red bar); **e** clear cell morphology; **f** nuclear p65-RelA; **g** case with homozygous *CDKN2A* deletion/loss of p16 protein (arrow, endothelial cell as internal positive control); **h** case without *CDKN2A* deletion/retained p16; **i–k** Kaplan–Meier analysis, impact of *CDKN2A* deletions on OS

presence of *CDKN2A* deletions (homo- or hemizygous) correlated with higher age at diagnosis in line with the literature [3, 5, 8]. *CDKN2A* deletion may also occur as secondary event in tumor progression [7].

To identify possible differences between RELA-EP with versus without *CDKN2A* deletion on the transcript level, 12 RELA-EP were analyzed by RNA sequencing for differentially expressed genes. After correction for multiple testing, five genes were found significantly downregulated including *CDKN2A* and *CDKN2B* and their neighboring gene *MTAP* (*S*-methyl-5′-thioadenosine phosphorylase) located in the deleted region. MTAP is a key enzyme in the methionine salvage pathway. Its deletion leads to dependence on the activity of the methyltransferase PRMT5 [6] which can be blocked by PRMT5 inhibitors as interesting novel therapeutics in *MTAP* deleted tumors. In addition, *KIF7* (15q26) encoding a cilia-associated protein and *ZNF536* (19q12) encoding a neuronal marker were found downregulated. *GABRA2* (4p12) encoding the gamma-aminobutyric acid receptor subunit alpha-2 was found highly upregulated in *CDKN2A* deleted tumors (supplementary figure 3).

Kaplan–Meier analysis revealed a significant correlation between *CDKN2A* deletions and overall survival status (OS). Different groups were compared: (1) homozygous *CDKN2A* deletion vs. hemizygous *CDKN2A* deletion and tumors with two retained alleles ($p = 0.009$); (2) homo- or hemizygous *CDKN2A* deletions vs. tumors with two retained alleles ($p = 0.034$) and (3) all three strata separately ($p = 0.017$) (Fig. 1i–k). In contrast to Korshunov et al. [5], neither homozygous nor hemizygous deletion showed prognostic relevance regarding EFS (supplementary figure 2). Predominant clear cell morphology as a histological feature was a favorable prognosticator for OS ($p = 0.039$), and high mitotic activity (> 17 mitoses/10HPF) was a predictor for tumor relapse ($p = 0.004$) as well as OS ($p = 0.007$) (supplementary figure 1). Multivariate analysis confirmed mitotic activity as independent prognostic indicator for EFS (supplementary table 2).

Our data show that deletions of *CDKN2A* represent an objective parameter for risk stratification in RELA-EP. Molecular inversion probe methodology turned out to represent a sensitive and quantitative tool for *CDKN2A* assessment in FFPE material. Apart from ependymoma, homozygous deletions of *CDKN2A* have recently been described as adverse prognostic marker for other CNS tumors, including anaplastic *IDH*-mutant gliomas and *BRAF*-mutant low-grade gliomas [1, 4, 11]. The deletion/inactivation of *CDKN2A* may result in a pathological activation of cyclin-dependent kinases 4/6 targetable by specific inhibitors such as palbociclib. Therefore, *CDKN2A* inactivation in RELA-ependymomas may represent a potential therapeutical target.

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