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

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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
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 *CI1orf95* (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-S'-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 prognostic factor 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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