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WNT-activated embryonal tumors of the pineal region: ectopic medulloblastomas or a novel pineoblastoma subgroup?

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Pineoblastoma (PB) is a CNS embryonal tumor of childhood with propensity to metastasize and a 5-year overall survival of 61% [5]. Originating from the pineal gland, PBs carry molecular profiles distinct from medulloblastomas (MBs), the most frequent embryonal tumors that by definition are located in the posterior fossa [2]. Based on methylation profiling, independent studies have identified biologically and clinically relevant subgroups in PB, driven by discrete genomic alterations [4, 5, 7]. In addition to tumors comprising these bona fide PB subgroups, rare outliers have anecdotally been described. Here, we present a multi-institutional series of patients (n=7) with WNT-activated embryonal tumors epicentered at the pineal region, including three unreported patients and four that had been briefly described [4, 5, 8]. Findings from DNA methylation array, tumor sequencing, and immunohistochemistry were reported. Clustering and copy-number analysis of methylation

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Data availability

Data from methylation profiling are available from the corresponding author upon request.

profiles were performed as previously described, adopting a radiographically and molecularly verified in-house cohort of MB and PB as reference [5].

Among the 7 patients, 4 were male, and the median age of diagnosis was 15 years (Supplementary Table 1). MRI revealed tumor centering at the pineal gland in 5 patients, and the posterior aspect of the thalamus abutting the third ventricle in 2 (Fig. 1a). No posterior fossa lesion was present. Six of the patients had localized disease, while 1 had a metastatic focus at the internal auditory canal (Supplementary Fig. 1a). Three patients underwent gross or near-total resection, 1 had subtotal resection and 3 had biopsy. Histology of all tumors were compatible with CNS embryonal tumors. Surprisingly, methylation profiling revealed that 6 samples clustered with WNT-activated medulloblastoma (MB-WNT), while 1 sample had no matching entity (Fig. 1b and Supplementary Fig. 2a). Monosomy 6 was observed in 3 patients and 6q loss in 1 (Supplementary Fig 2b). Tumor sequencing revealed missense mutations in exon 3 of *CTNNB1* in all samples (Fig. 1c and Supplementary Table 1). Orthogonal validation by immunohistochemistry (n=4) confirmed nuclear beta-catenin accumulation and positive LEF1 staining, indicative of WNT-pathway activation (Fig. 1d). All patients underwent adjuvant treatment based on regimens for CNS embryonal tumors (Supplementary Table 1). In particular, craniospinal irradiation without primary site boost was delivered in 2 patients (Patients 4, 5), reduced-dose craniospinal irradiation (23.4Gy) and boost in 1 (Patient 3), and focal radiotherapy in 1 (Patient 2). At median follow-up of 3 years (range: 0.8-12.8), all patients were alive without disease (n=5) or with stable residual disease (n=2). Rapid response to treatment was observed in patients who received biopsy only rendering second-look surgery unnecessary (Supplementary Fig. 1b).

Activation of the WNT-pathway is implicated in the initiation and propagation of cancers [1]. The exact mechanism leading to pathway overactivity and corresponding prognostic significance is disease-specific. Mutations in exon 3 of *CTNNB1* that disturb physiological phosphorylation and degradation of CTNNB1, represent a hallmark feature of MB-WNT, and in turn a predictor for good outcome [3]. Analogous to the pathogenesis of MB-WNT from the lower rhombic lip [6], the anatomical-molecular profile of tumors in our report might be the result of *CTNNB1* mutations transforming neural stem cells in the pineal region and/or third ventricle subventricular zone. Overlapping DNA methylation signatures and immunohistochemical patterns with WNT-activated MBs may suggest that the definition of MB needs to be adapted to reflect rare pineal region diagnoses. Overall, our study demonstrates that adequate molecular work-up of pineal region tumors is invaluable to detect tumor drivers of prognostic significance, and may allow de-escalation of therapy in an appropriate subset of patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. (a) Diagnostic MRI (mid-sagittal, axial) depicting the presence of pineal region tumors. (b) t-SNE representation of methylation profiles demonstrates clustering of 6/7 study samples with WNT-activated medulloblastoma (MB-WNT). (c) Summary of *CTNNB1* mutations in study samples with reference to those identified in MB-WNT (PeCan database, St. Jude Children's Research Hospital). (d) Histopathologic features by H&E staining and immunohistochemistry (120x, scale bar=120μm), showing small cell embryonal tumor, nuclear accumulation of beta-catenin in a proportion of tumor cells, and diffuse positive staining for LEF1. MB-G3, Group 3 medulloblastoma; MB-G4, Group 4 medulloblastoma; MB-SHH, SHH-activated medulloblastoma; PB, pineoblastoma (subgroup annotation according to Liu et al. [5]); Pin-WNT, pineal region tumors with WNT-activation