



## Nucleolar immunohistochemical expression of H3K27me3 in a pediatric cerebellar lesion: A true or false positive?

A 3.4-year-old boy presented with nocturnal vomiting, ataxia, and dysarthria. MRI revealed a cerebellar lesion (85 mm × 80 mm × 80 mm) suggestive of an aggressive embryonal tumor. After neurosurgery with complete resection, histology showed a proliferation of round hyperchromatic cells with perivascular pseudorosettes, discohesive cells, and pseudopapillae. Immunohistochemistry (performed using the BenchMark Ultra automated immunostainer -Ventana Medical Systems, Roche Diagnostics Division, Hoffman La Roche Ltd, Basel, Switzerland- and always performed with a positive control according to the correct international quality standard) was negative for glial fibrillary acidic protein (GFAP) but positive for synaptophysin and  $\beta$ -catenin (both cytoplasmic). The cell proliferation index (Ki67) was very high, and nuclear sal-like protein 4 (SALL4) positivity was noted [Figure 1]. Interase interactor 1 (INI1) and Lin-28 homolog A (LIN28A) were positive (nuclear and cytoplasmic, respectively). Interestingly, Histone H3 trimethylation at lysine 27 (H3K27me3) showed nucleolar positivity at high magnification in pseudonodular areas of high cell density [Figure 2]. Molecular studies excluded C19MC and DICER1 mutations with a wildtype profile (C19MC<sup>wt</sup>/DICER1<sup>wt</sup>), but the methylation profile led to the diagnosis of embryonal tumor with multilayered rosettes (ETMR), grade 4 according to the WHO 2021 classification of central nervous system tumors. Despite chemotherapy and autologous stem cell transplantation, the patient died 8 months after diagnosis.

ETMRs represent a rare and aggressive subtype of pediatric embryonal brain tumors. Histologically, ETMRs are classified into three variants: embryonal tumor with abundant neuropil and true rosettes, ependyoblastoma, and medulloepithelioma. Beyond the histological pattern, the vast majority of ETMRs (90%) harbor alterations in the C19MC microRNA cluster (C19MC<sup>altered</sup>), with a smaller subset (5%) harboring mutations (somatic or germinal), in the DICER1 (DICER1<sup>mut</sup>) gene (a ribonuclease III enzyme that is essential for the biogenesis of microRNAs). The remaining cases (5%) represent a heterogeneous group harboring very rare and not fully known molecular alterations; this last group is recommended to be classified as ETMR “not elsewhere classified” by WHO-CNS2021 [1].

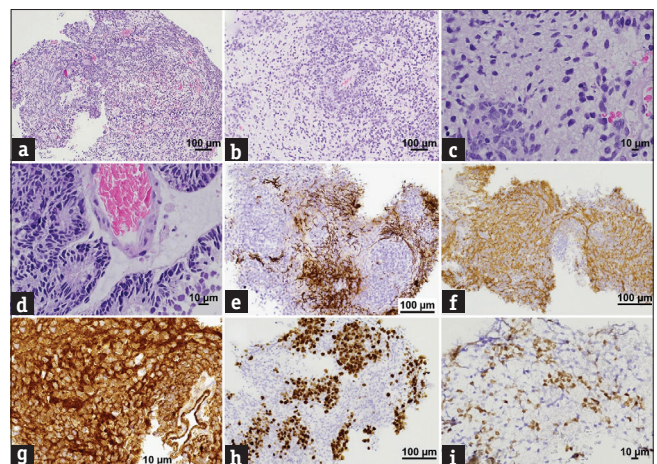
One of the major challenges in treating ETMRs is their aggressive nature and poor prognosis, largely due to the difficulty in achieving complete surgical resection, as these tumors are often deeply infiltrative and located in critical areas of the brain. In addition, while high-dose multi-agent chemotherapy has shown some benefit, it is associated with significant dose-limiting toxicities and radiation therapy is generally contraindicated in infants due to its adverse neurodevelopmental effects. The rarity of ETMRs also makes it difficult to conduct large-scale clinical trials, limiting the availability of evidence-based treatment protocols. In

addition, the presence of C19MC amplification in ETMR adds another layer of complexity, as targeted therapies for this specific genetic alteration are still in the experimental stage, making precision medicine for this tumor type a significant challenge [2].

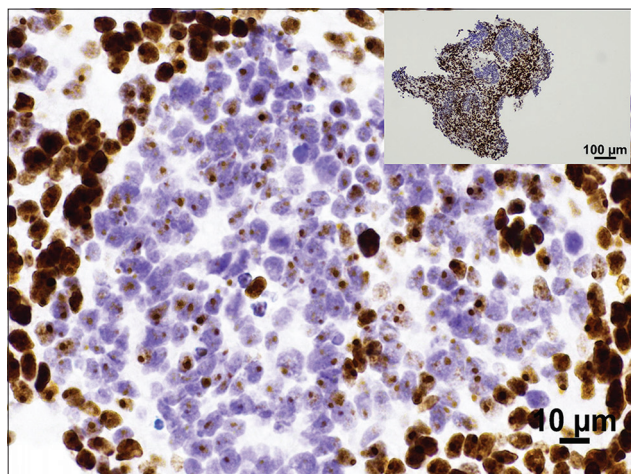
Diagnostic criteria for ETMR include negative staining for GFAP, oligodendrocyte transcription factor 2, and epithelial membrane antigen and positive for synaptophysin,  $\beta$ -catenin, INI1, and LIN28A. LIN28A, a regulator of let-7 microRNAs, plays a key role in promoting cell proliferation and inhibiting apoptosis, linking miRNAs to tumor pathogenesis. Recently, some ETMRs DICER1<sup>mut</sup> have also been shown to be associated with H3.3K27M mutation and loss of H3K27me3 immunostaining, i.e., the same molecular pattern (H3.3K27M<sup>mut</sup>/H3K27me3<sup>-</sup>) observed in midline gliomas [3,4].

The present case deviates from these typical molecular profiles. It falls within the 5% of ETMRs that are wildtype for both C19MC and DICER1 but presents a novel molecular profile: LIN28A<sup>+</sup>/SALL4<sup>+</sup>/H3K27me3<sup>nucleolar+</sup>/H3.3K27M<sup>-</sup>/C19MC<sup>wt</sup>/DICER1<sup>wt</sup>, which distinguishes it from other known ETMR subtypes.

Nucleolar positivity for H3K27me3 is significant. H3K27me3 is a marker of heterochromatin, a form of tightly packed chromatin involved in gene silencing. While the nucleolus is primarily responsible for ribosome biogenesis, it also contains heterochromatin, so the presence of H3K27me3 in the nucleolus is consistent with its known location. This observation supports the validity of our immunohistochemical findings and raises interesting therapeutic possibilities. Indeed,



**Figure 1:** (a-d) Photomicrographs showing different aspects of the neoplasm: in (a) diffuse, in (b) perivascular distribution, in (c) areas of abundant interposed glial tissue, and in (d) focal pseudopapillary architecture. (e) Immunohistochemistry (IHC) for glial fibrillary acidic protein showing expression in the interstitial glial areas but negativity in the neoplasm. (f) Positive IHC for synaptophysin. (g) Positive IHC for beta-catenin. (h) IHC for Ki67 with very high cell proliferation index in pseudonodular areas of neoplastic aggregation. (i) IHC for sal-like protein 4 (SALL4) with nuclear positivity



**Figure 2:** Photomicrograph showing that in the neoplastic areas corresponding to those of greatest proliferation (see Ki67 expression in Figure 1h above), there is an apparent loss of H3K27me3 expression when the tissue is examined at low magnification, but instead corresponds to nucleolar expression at very high magnification ( $\times 60$ ). Noteworthy, the nuclear expression of H3K27me3 is correctly maintained in the internodular areas, which also provides a positive internal control

it opens a reflection on the fact that the methylation of histone H3 at lysine 27 (H3K27me3) is catalyzed by the Polycomb Repressive Complex 2 (PRC2) and that the Enhancer of Zeste Homolog 2 (EZH2) is a core component of PRC2 and plays a critical role in the establishment and maintenance of repressive chromatin domains. Interestingly, EZH2 has emerged as a promising therapeutic target in various tumors, including those with aberrant H3K27me3 profiles. Given the unique H3K27me3 profile in our case, it is tempting to speculate that targeting EZH2 could be a potential therapeutic strategy for ETMRs with similar characteristics [5].

Another intriguing aspect is the expression of sal-like protein 4 (SALL4), a key regulator of stem cell pluripotency and self-renewal. SALL4 is involved in gene silencing by interacting with epigenetic modifiers and associating with heterochromatin. While SALL4 is known as an “embryonic marker,” its association with ETMRs outside of DICER1 mutations has not been reported. Our case is the first to describe SALL4 expression in association with H3K27me3 nucleolar positivity: This could be a valuable finding as SALL4 is emerging as a target for therapeutic strategies, although to date there is no conclusive data on the use of SALL4-targeting molecules in DICER-associated SALL4+ tumors, let alone in ETMR SALL4+ [6]. The mechanisms and/or pathways underlying our findings are not yet understood, but the morphological description of the observations may stimulate detailed molecular investigation, providing new insights into the pathogenesis of ETMR.

#### Ethics statement

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and its amendments. The authors certify that they have obtained appropriate consent form from the legal guardians of the patient. In the form, the guardians have given the consent for the images and other clinical information of the patient to be reported in the journal. The guardians understand that the name and initials of the patient will not be published and

due efforts will be made to conceal the identity, but anonymity cannot be guaranteed.

#### Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

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Nil.

#### Conflicts of interest

There are no conflicts of interest.

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#### REFERENCES

1. WHO Classification of Tumours Editorial Board. Central nervous system tumours. WHO classification of tumours series. 5<sup>th</sup> ed., Vol. 6. Lyon: International Agency for Research on Cancer; 2021.
2. Bandopadhyay P, Chi SN. The challenges in treating embryonal tumors with multilayered rosettes (ETMR) and other infant brain tumors. *Neuro Oncol* 2022;24:138-40.
3. Rossi S, Barresi S, Stracuzzi A, Lopez-Nunez O, Chiaravalli S, Ferrari A, et al. DICER1-associated malignancies mimicking germ cell neoplasms: Report of two cases and review of the literature. *Pathol Res Pract* 2021;225:153553.
4. Wang L, Lu D, Piao Y. A 2-year-old girl with posterior fossa mass. *Brain Pathol* 2022;32:e13026.
5. Sabour-Takanlou M, Sabour-Takanlou L, Biray-Avci C. EZH2-associated tumor malignancy: A prominent target for cancer treatment. *Clin Genet* 2024;106:377-85.
6. Moein S, Tenen DG, Amabile G, Chai L. SALL4: An intriguing therapeutic target in cancer treatment. *Cells* 2022;11:2601.

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