Brief Report
Metastatic Group 3 Medulloblastoma in a Patient With Tuberous Sclerosis Complex: Case Description and Molecular Characterization of the Tumor

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Medulloblastoma is the most common pediatric brain tumor. We describe a child with tuberous sclerosis complex that developed a Group 3, myc overexpressed, metastatic medulloblastoma (MB). Considering the high risk of treatment-induced malignancies, a tailored therapy, omitting radiation, was given. Based on the evidence of mammalian target of rapamycin mTORC, mTOR Complex; RAS, Rat sarcoma; RAF, rapidly accelerated fibrosarcoma (mTOR) pathway activation in the tumor, targeted therapy was applied resulting in complete remission of disease. Although the PI3K/AKT/mTOR signaling pathway plays a role in MB, we did not find TSC1/TSC2 (TSC, tuberous sclerosis complex) mutation in our patient. We speculate that a different pathway resulting in mTOR activation is the basis of both TSC and MB in this child; H&E, haematoxilin and eosin; Gd, gadolinium. Pediatr Blood Cancer © 2015 Wiley Periodicals, Inc.

Key words: medulloblastoma; mTOR; TSC1/2

INTRODUCTION

Medulloblastoma (MB) is the most common malignant central nervous system (CNS) tumor of childhood. Current multimodal treatment of MB has led to a 5-year overall survival of about 87% for average-risk and 70% for high-risk patients.[1] However, prognosis remains poor for patients with recurrent MB. Recently, four molecular subgroups have been described (wingless/integrated [WNT], sonic hedgehog [SHH], Group 3, and Group 4) that differ in their demographics, transcriptomes, somatic genetic events and clinical outcomes.[2]

CNS malignancies can be associated with different neurogenetic syndromes, including tuberous sclerosis complex (TSC). TSC is an autosomal dominant neurocutaneous disease affecting about one in 6,000 newborns, and characterized by the presence of widespread hamartomas. CNS involvement results in a high rate of epilepsy, mental retardation, and neuropsychiatric disorders.[3] This disease has a variable expression ranging from severe mental retardation, intractable seizures, and extensive organ involvement to patients presenting few and asymptomatic hamartomas, normal cognitive levels, and no seizures. Mammalian target of rapamycin (mTOR) overactivation is the key finding in TSC.[4] Experimental findings suggest that inactivation of *TSC* genes can cooperate with SHH signaling to drive tumor growth through mTOR-dependent translation machinery.[5] Although patients with TSC are at increased risk of developing CNS malignancies,[6] an association with MB has never been described.

RESULTS

Our patient was born at term after the first uneventful pregnancy to healthy unrelated parents. At 14 months of age, she began to present focal seizures. Magnetic resonance imaging (MRI) revealed the presence of several cortico-subcortical tubers associated with small subependymal lesions, leading to a definite diagnosis of TSC according to revised diagnostic criteria.[7] Further investigation revealed the presence of a single ash-leaf hypomelanotic macule and cardiac rhabdomyomas.

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Seizure control was obtained by carbamazepine. Normal psychomotor development was observed thereafter. Molecular genetic analysis on peripheral blood cells, including next-generation sequencing and multiplex ligation-dependent probe amplification, revealed no mutations.

At the age of 5 years and 7 months, she presented with torticollis associated with headache, vomiting, and ataxia. MRI showed a large rounded cerebellar lesion, arising from the vermis and occupying the fourth ventricle with secondary hydrocephalus. A second mass was observed in the pituitary peduncle (Fig. 1A).

After endoscopic third ventriculostomy, subtotal resection was performed with residual disease on the floor of the fourth ventricle. Cerebro-spinal fluid sampling confirmed dissemination of disease. A postoperative posterior fossa syndrome was observed from which the patient had completely recovered at 40 days from surgery.

Histology documented a large cell/anaplastic (LC/A) MB (Fig. 1B). Overexpression of NRL (neural retina leucine zipper), NRP3, MAB21L2 (mab-21-like 2), IMPG2 (interphotoreceptor matrix proteoglycan 2), EGFL11 (alias of EYS: eyes shut homolog), and GABRA5 (gamma-aminobutyric acid [GABA] A receptor, alpha 5) was suggestive of a molecular Group 3 MB (Fig. 2A). *MYC* was not amplified but messenger RNA expression levels of both c-myc and n-myc were higher than in normal cerebellar tissue (Fig. 2B).

Western blot analysis showed strong expression of mTOR and its phosphorylated form (Fig. 2C). No mutations were identified in the MB, thus excluding the presence of *TSC1* and *TSC2* somatic mutation in the tumor.

The patient was treated with a tailored chemotherapeutic regime based on methotrexate, cyclophosphamide, and carboplatin plus vincristine. Complete remission of disease was obtained and two courses of high-dose thiotepa with autologous peripheral hemopoietic stem cell rescue were administered. Considering the pivotal role of mTOR in the pathogenesis of TSC-associated tumors, off-label treatment with everolimus, a selective inhibitor of mTOR pathway, was started. Radiotherapy was discussed but not performed and the patient is alive and in complete remission of the disease at 17 months from diagnosis. Antiepileptic treatment was gradually stopped without any electroencephalogram (EEG) evidence of residual epileptic activity.
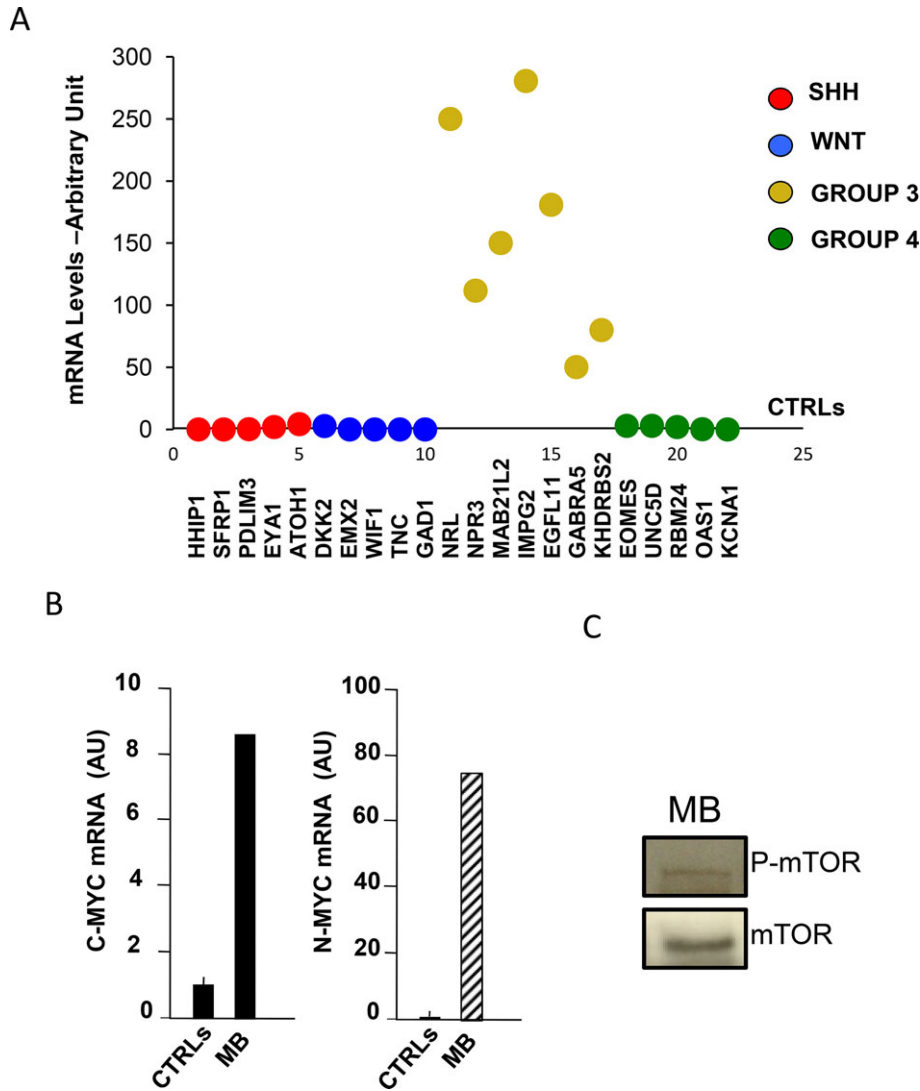
DISCUSSION

MB is the most common malignant brain tumor in children and, despite recent treatment advances, approximately 40% of children experience tumor recurrence and 30% will die of their disease. Molecular profiling of MB has recently emerged as a tool for risk stratification with molecular subgroup 3 having the worst prognosis.[1] Group 3 tumors account for 28% of all MBs, have a high incidence of LC/A histology and are very frequently metastatic. LC/A MB are characterized by c-myc overexpression, which is highly associated with a worse clinical outcome.[1] Our patient presented several poor prognosis indicators, includ-
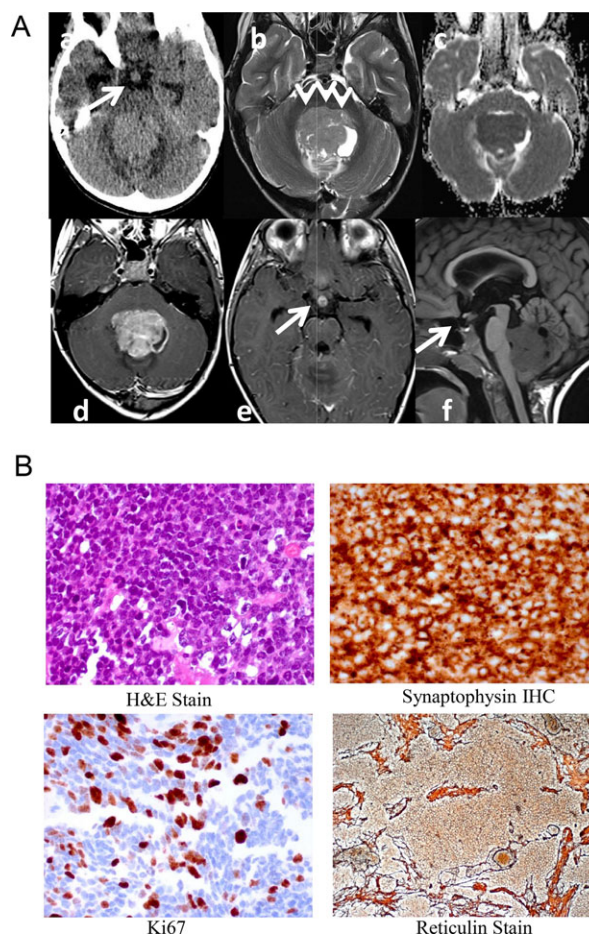
Fig. 1. (A) Axial CT scan (a); axial T2-weighted image (b); ADC map (c); Gd-enhanced axial T1-weighted images (d and e); Sagittal T1-weighted image (f). The midline mass is slightly hyperdense to brain on CT scan (a). The lesion is slightly hyperintense on T2-weighted images (b). Diffusion weighted imaging—ADC map (c) showing areas of restricted diffusion due to the high cellularity/high nucleus–cytoplasm ratio. Marked and inhomogeneous enhancement is seen following gadolinium administration (d). Axial image shows thick neoplastic infiltration of the fourth ventricular floor (arrow heads, b). The sagittal T1-weighted image (d) shows that the hypointense lesion arises from the vermis and fills the fourth ventricle. There is gross dilatation of the superior part of the fourth ventricle and supratentorial hydrocephalus. Note a secondary lesion at the level of the infundibulum (arrow; a, e, and d), and downward tonsillar displacement (f). (B) Highly cellular neoplasia composed of round small/medium-sized cells, with hyperchromatic nuclei and scanty cytoplasm. Nuclear polymetrism and atypical mitoses are evident (H&E stain); diffuse pattern of synaptophysin positivity of neoplastic cells; high proliferation rate (Ki67 IHC stain). Reticulin stain revealed/showed a diffuse pattern of growth without pale area formation or nodularity features.

ing LC/A histology, molecular subgroup 3, metastatic disease, and residual disease after surgery.

Although MBs are more often sporadic, they can be found in different syndromic conditions such as Gorlin syndrome, familial adenomatous polyposis, Fanconi anemia, RASopathies, Rubinstein–Taybi syndrome, Mowat–Wilson syndrome, and Turner syndrome.[8–12] The most frequent CNS tumors in TSC

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Fig. 2. (A) MB molecular characterization and c-MYC expression—messenger RNA (mRNA) levels of the indicated genes in MB compared to normal cerebellum (average, n = 8) as control (CTRLs). Genes are grouped and depicted in different colors, depending on the molecular subgroup, which are identified as SHH, WNT, Group 3, and Group 4. The values of relative quantification are expressed in linear scale arbitrary units. (B) Histograms showing mRNA expression levels of c-MYC (left panel) and n-MYC (right panel) in MB compared to normal cerebellum (average, n = 8) as CTRLs. (C) MB tissue samples were lysed in Ripa buffer and resolved by 8% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Western blotting anti-P-mTOR was performed to evaluate mTOR activation.

are subependymal giant cell astrocytomas (SEGA), CNS malignancies that are exceedingly rare and limited to glioblastoma multiforme.[6,7] To our knowledge, this is the first report of MB associated with TSC.

As regards any tumor predisposing condition, we speculate that the genetic substrate of TSC might have played a role in the onset of the MB in our patient. TSC commonly results from mutations in the *TSC1* or *TSC2* genes encoding hamartin and tuberin, respectively.[13] Ten to 25 percent of patients affected by TSC have no mutation identifiable by available genetic testing, and therefore in these cases a definite diagnosis is made using clinical diagnostic criteria.[7]

Independent of mutational status, hamartin/tuberin dysfunction is a central event in TSC pathogenesis, leading to overactivation of the mTOR pathway that is responsible for the formation of hamartomas and malignancies typical of TSC.[14–16] Notably, *TSC2* mutation has also been reported in MB samples and, more recently, *TSC1* mutation has been detected in Group 3 MB.[2,17] RAS/RAF-mediated m-TORC activation has also been described in MB with resulting c-MYC translational regulation conditioning cell growth and proliferation.[18,19]

Our patient showed normal expression of *TSC1* and *TSC2* both on blood samples and tumor tissue. However, mTOR was activated as shown by its phosphorylated status, and c-MYC was overexpressed with no evidence of amplification.

We hypothesize a key role for *TSC1/TSC2*-independent activation of mTOR in the pathogenesis of both TSC and MB. Of note, our patient showed a surprising sensitivity to chemotherapy with complete remission of disease after only two treatment courses without radiation therapy. Beginning treatment early

after surgery (15 days), when the blood–brain barrier had not recovered, might have played a role in maximizing response to treatment. Despite the well-documented role of radiation in the treatment of MB, we decided to omit it considering the dramatic response to chemotherapy and the risk of secondary malignancies due to her cancer predisposition syndrome.

Based on the previous reports for SEGAs, our child received a dose of 3 mg/m^2 of everolimus (range 5–15 ng/ml), and we speculate that this treatment might have contributed to remission of disease. Moreover, although no specific effect on epilepsy can be inferred as clinical seizures had not been observed for a long time before tumor diagnosis, our patient had a complete normalization of her EEG 6 months after treatment initiation.[20]

In conclusion, patients affected by TSC should be considered at risk for CNS malignancies, and the posterior fossa should be considered in all neuroradiologic examinations. MB associated with TSC may have a different behavior from comparable sporadic MB with a more favorable outcome. Indications for radiation therapy should be considered on an individual basis, especially in the presence of a dramatic response to chemotherapy. Considering the pivotal role of mTOR activation in TSC, it should be tested in all associated malignancies to guide targeted therapy.

ACKNOWLEDGMENTS

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