

## Brain lesions in tuberous sclerosis complex. Review

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### Abstract

*Tuberous sclerosis complex (TSC) is an autosomal dominant, multisystem disease characterized by the development of multiple hamartomas and benign or rarely malignant neoplasms distributed at various sites throughout the body, especially in the brain, skin, retina, kidney, heart, and lungs. Brain lesions in TSC include: cortical/subcortical glioneuronal tubers, subependymal glial nodules (SENs), and subependymal giant cell astrocytomas (SEGAs). Cortical tubers are characterized by a markedly disorganized cortical lamination with dysplastic aggregates of abnormal glial and neuronal elements, including giant cells. SENs consist of large cells, somewhat similar to the giant cells seen in tubers, accompanied by elongated glial cells. SENs are typically covered by a layer of ependyma and can grow over time and develop into subependymal giant cell astrocytomas. SEGAs consist of a mixed cell population of large ganglioid-like cells, spindle and giant cells with nuclear pleomorphism. Mitotic activity and necrosis might be observed in SEGAs but they should not be considered as features of malignancy. The clinical presentations of TSC result from mutations in either of two tumour suppressor genes: TSC1 (located on 9q34) or TSC2 (located on 16p13). The proteins encoded by TSC1 and TSC2 genes, hamartin and tuberin, respectively, form a heterodimer which suppresses the mammalian target of rapamycin (mTOR), a major cell growth and proliferation controller. Oral rapamycin therapy may induce regression of astrocytomas associated with TSC. In this review, the clinicopathological features of TSC and recent advances in the diagnosis and genetics of TSC are presented.*

**Key words:** TSC, SEGA, cortical tubers, SEN, hamartin, tuberin, mTOR, rapamycin therapy.

### Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant, multisystem disease with high penetrance and variability. Its incidence is estimated to be as high as 1 : 6000 [1,6,7]. TSC is characterized by the development of multiple hamartomas and benign or rarely malignant neoplasms distributed at various sites throughout the body, especially in the brain, skin, retina, kidney, heart, and lungs [6,7,14,15,18]. Cuta-

neous lesions include angiofibromas, localized cutaneous thickenings (shagreen patches), hypopigmented macules ("ash-leaf" patches) or spots (confetti-like lesions) and ungual or periungual fibromas known as Koenen's tumours. Brain lesions in TSC include: cortical/subcortical and subcortical tubers, subependymal nodules (SEN), and subependymal giant cell astrocytomas (SEGA), as well as white matter lesions [24,30]. The individuals with TSC can also develop renal and

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liver angiomyolipoma, renal clear cell carcinoma, retinal glial hamartomas, cardiac rhabdomyomas and pulmonary lymphangioleiomyomatosis (LAM) or multifocal micronodular hyperplasia of type II pneumocytes [26]. Cysts may be found in various locations, including the liver, kidneys and pancreas [17,27]. Various vascular lesions, including aneurysms may be found in brain and other locations in TSC patients [14,15]. In 1998, the specific clinical diagnostic criteria for TSC were established and divided into two main categories: major and minor (Table I) [26]. In children with TSC, the brain lesions are the major causes of morbidity and mortality. The majority of TSC patients present with epilepsy, beginning in early childhood, cognitive impairment, autism spectrum disorders, and sleep disorders [18].

### Radiologic features of brain lesions

Brain lesions in TSC include cortical tubers, subependymal nodules, radial hypomyelinated tracts

extending from subependymal area to the cortex, and subependymal giant cell astrocytomas (SEGAs). The MRI pattern of these lesions changes with the age of patients.

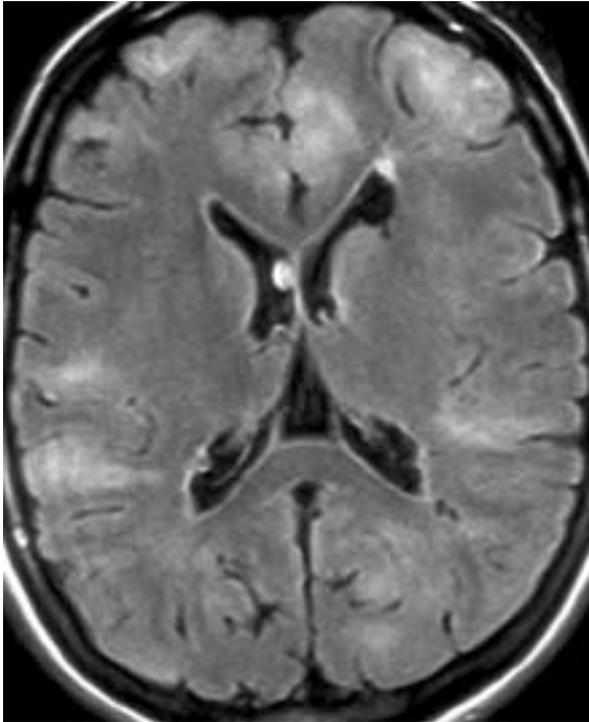
### Cortical tubers

They are mostly supratentorial, although 8-15% of patients with TSC develop infratentorial cerebellar tubers. In neonates and young infants cortical tubers have high signal on T1-weighted images (WI) and low on T2-WI compared to the unmyelinated white matter [2]. In older children and adult patients the tubers appear hypointense on T1-WI and hyperintense on T2-WI and FLAIR images (Fig. 1). Most of the affected gyri are enlarged. Some of them present cyst-like changes: FLAIR sequences typically show central signal loss with a high signal peripheral rim [17,27]. The cause of the cystic changes is unknown but these changes might reflect cellular degeneration. It is more commonly seen in children younger than 7 years.

**Table I.** Revised diagnostic criteria for TSC (according to Roach *et al.* 1998).

Definite TSC diagnosis is established, when either two major features or one major feature plus two minor features are present. Probable TSC diagnosis is established, when one major plus one minor feature is present, and possible TSC diagnosis is established, if either one major feature or two or more minor features are seen in a patient.

Major features	Minor features
Facial angiofibromas or forehead plaque	Multiple, randomly distributed pits in dental enamel
Nontraumatic ungual or periungual fibroma	Hamartomatous rectal polyps
Hypomelanotic macule (3 or more)	Bone cysts: radiographic confirmation is sufficient
Shagreen patch (connective tissue nevus)	Cerebral white matter radial migration lines
Multiple retinal nodular hamartomas	Gingival fibromas
Cortical tubers	Nonrenal hamartomas
Subependymal nodule	Retinal achromic patch
Subependymal giant cell astrocytoma	“Confetti” skin lesions
Cardiac rhabdomyoma, single or multiple	Multiple renal cysts
Lymphangioleiomyomatosis: when both lymphangioleiomyomatosis (LAM) and renal angiomyolipomas (AMLs) are present, other features of tuberous sclerosis should be present before a definite diagnosis is assigned	
Renal angiomyolipoma: when both LAM and renal AMLs are present, other features of tuberous sclerosis should be present before a definite diagnosis is assigned (see previous remarks)	



**Fig. 1.** Cerebral cortical tubers – axial FSE FLAIR image. Typical hyperintense signal of multiple cortical tubers.



**Fig. 2.** Subependymal nodules – axial T1-weighted image after gadolinium injection. Ring-like pattern of enhancement of subependymal nodules.

About half of cortical tubers calcify, which is well seen on CT images. On MR images, the large calcified lesions show significant signal loss on T2-WI and T2\*WI (gradient echo sequences) [4]. Calcification incidence also increases with age, it is rare in infants. The tubers exhibit contrast enhancement in approximately 3-4% of cases. Unlike cortical tubers, the cerebellar tubers are usually wedge-shaped.

Proton spectroscopy of tubers shows a decrease of N-acetylaspartate (NAA) and increased myo-inositol, which confirms a reduction of neurons, gliosis, or the presence of immature neurons [2].

### Subependymal nodules

Subependymal nodules occur along the ventricular surface but they are most commonly located near the caudate nucleus just behind the foramen of Monro. On MR images, SENs appear as irregular nodules, signal is variable, usually hypointense to white matter on T2, because of calcification [2,4]. In neonates, these lesions are typically not calcified and appear

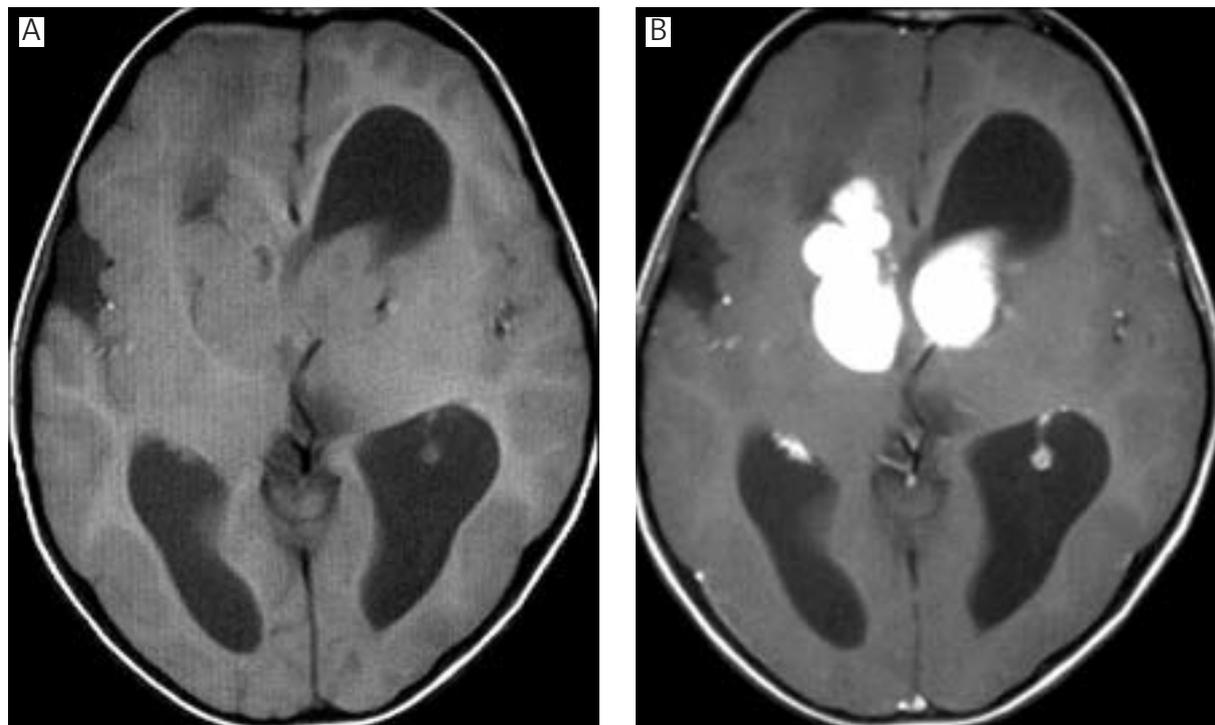
hyperintense in comparison to unmyelinated white matter on T1-WI. Calcifications might increase with age. Variable enhancement of subependymal nodules are seen after contrast injection, but some nodules have no enhancement (Fig. 2).

### Subependymal giant cell astrocytomas

SEGAs are located at or near the foramen of Monro (Fig. 3A). SEGAs are frequently calcified, and have heterogenous signal on MR images [3]. After contrast administration, these lesions show intense but inhomogenous enhancement (Fig. 3B). SEGAs also enlarge with the time. Associated obstructive hydrocephalus is common.

### Histopathologic features

The main neuropathologic features of TSC include cortical and subcortical tubers, subependymal nodules (SENs), and subependymal giant cell astrocytomas (SEGAs) [18,24,30].



**Fig. 3.** SEGA – axial T1-weighted images. **A)** Bilateral large isointense masses at the foramen of Monroe before gadolinium injection, **B)** The strong and homogenous enhancement seen after gadolinium injection.

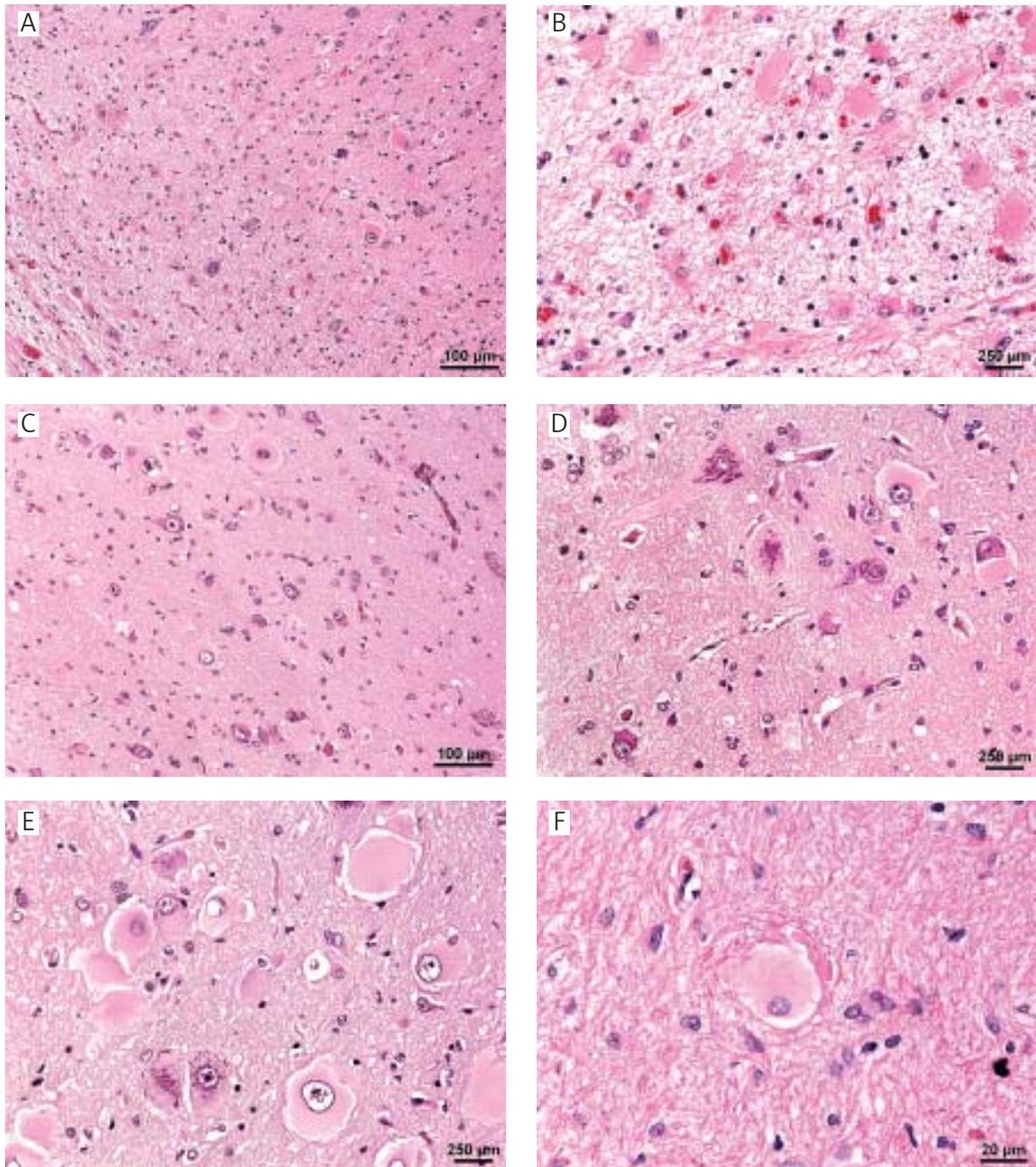
## Tubers

Tubers are focal abnormalities of cortical architecture associated with TSC. Cortical tubers are found in the cerebral and cerebellar cortex, and subcortical white matter. Macroscopically, tubers are well-circumscribed, firm, pale, flat regions of cerebral cortex. They tend to be confined to a single gyrus or may span two or more gyri. In some cases, they may have lobar or even hemispheric distribution, sometimes even resulting in hemimegalencephaly. Tubers have a variable appearance with mushroom-shaped gyri and loss of the cortex-white matter junction. They are firmer and more pale than surrounding cortex or may exhibit irregular thickening and blurring of the cortical mantle and gray and white matter junction. Tubers can extend from the cortical surface deep into the subcortical white matter or can be confined to the cortical mantle. Their sizes range from a centimeter to several centimeters. Tubers may calcify or undergo cystic degeneration.

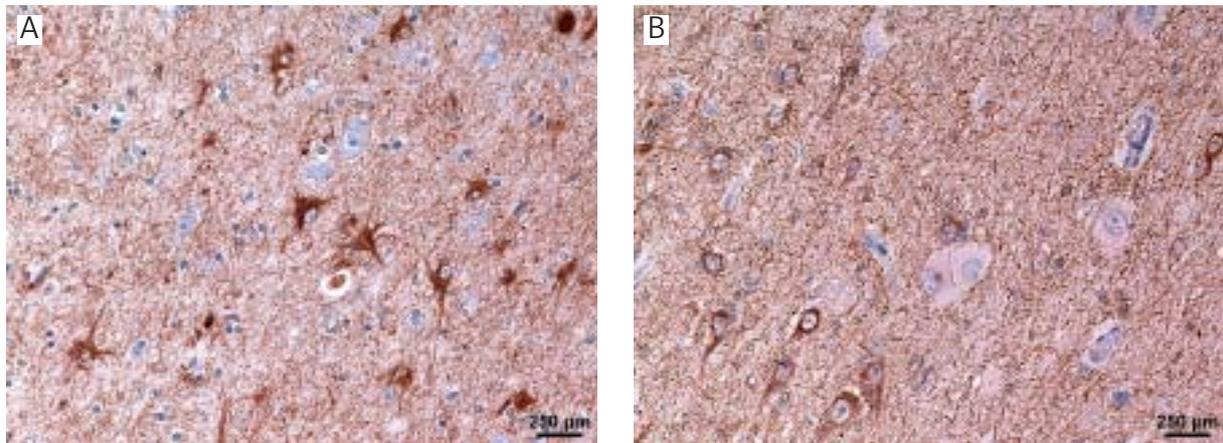
Microscopically, tubers are characterized by a marked disorganization of cortical lamination with aggregates of abnormal glial and dysplastic neuronal elements including giant cells [24,30] (Fig. 4A). Typi-

cally, the normal hexalaminar architecture of the cortex is lost or persists in only a rudimentary fashion. Other histologic features, often encountered, include vascular calcinosis with large aggregates of calcium distributed throughout the specimen.

The glial element consists of astrocytes of different size. Some enormous gemistocytic astrocytes with abundant, eosinophilic cytoplasm may be found (Fig. 4B). The tubers contain a heterogeneous population of neurons, some exhibiting bizarre and aberrant morphology, while others appear like normal cortical neurons (Fig. 4C). Dysmorphic neurons often show loss of radial orientation with respect to the pial surface, and exhibit an enlarged cell body with conspicuous Nissl substance (Fig. 4D). These dysmorphic neurons may reveal cytoskeletal abnormalities that are almost identical to those seen in the neurofibrillary tangle-containing neurons [30]. The tubers contain also cells of indeterminate neuronal versus glial phenotype with nucleated nucleus and glassy amphophilic or eosinophilic cytoplasm (Fig. 4E). The most characteristic feature of tubers is the presence of enlarged cells, known as “balloon cells” (BCs) or “giant cells” (GCs) (Fig. 4F) [18,24,30]. These cells are similar to BCs found



**Fig. 4.** Histopathology of cortical tuber. **A)** Cortex exhibiting disorganized cortical lamination with abnormal glial and dysplastic neuronal elements, H&E; **B)** Gemistocytic astrocytes with abundant, eosinophilic cytoplasm, H&E; **C)** Heterogenous population of neurons containing neuronal cells of normal and aberrant morphology, H&E; **D)** Dysmorphic neurons with enlarged cell body and conspicuous Nissl substance, H&E; **E)** Cells of indeterminate neuronal versus glial phenotype with nucleotated nucleus and glassy eosinophilic cytoplasm, H&E; **F)** Enlarged “balloon cell” (BC), H&E.



**Fig. 5.** Immunohistochemistry of cortical tuber. **A)** Strong GFAP immunoreactivity; **B)** Neurofilament immunoreactivity in dysmorphic neurons.

in sporadic focal cortical dysplasia (FCD) type 2B [11]. The maximal dimension of these cells is about 120 µm, but usually they range from 80 to 100 µm. GCs can be seen across the thickness of the tuber as well within the subcortical white matter or in the layer I. The dysmorphic neurons are interspersed with GCs throughout the thickness of the tuber. GCs can be observed as the single cells or they are clumped together in clusters or chains, often near small vessels. Some of the GCs are multinucleated.

Cortical tubers are rarely found in the cerebellum. The cerebellar tubers exhibit marked disorganization of neuronal architecture with ectopic neurons in the molecular and granule cell layers and white matter. Giant cells, calcifications, gliosis and Rosenthal fibers could be also detected.

### Immunohistochemistry

The glial cells exhibit the immunoreexpression of glial fibrillary acidic protein (GFAP) across the thickness of tubers (Fig. 5A). Increased expression of the adhesion molecule CD44 is also observed. The recent studies have demonstrated enhanced numbers of CD68 immunoreactive microglial cells within tubers. Dysmorphic neurons express glutamate transporters, such as EAAC1, and neuronal markers i.e. neurofilament protein (Fig. 5B) or microtubule associated protein-2 (MAP2) and SMI32.

GCs are a highly heterogenous population with some extending MAP2 positive dendrites while others extend several small neurofilament positive processes, suggestive of axons. GCs are periodic-acid Schiff (PAS) positive [28].

The variable immunophenotype of cells creating cortical tubers suggests that they are atypical astrocytes, atypical neurons, or cells of indeterminate origin [24,28].

### Subependymal nodules (SENs)

Subependymal nodules are present in about 80% of TSC patients and they are located around the wall of the lateral and third ventricle. These lesions develop in fetal life and often degenerate or calcify during later life.

Macroscopically, SENs are nodular lesions, less than 1 cm in size. They occur singly or in rows (candle guttering) and sometimes they are completely calcified.

Microscopically, SENs consist of large cells, somewhat similar to the giant cells seen in tubers, and elongated glial cells. SENs are typically covered by a layer of ependyma and can exhibit extensive vascularization. SENs can grow over time and develop into subependymal giant cell astrocytomas [18,24,30].

### Immunohistochemistry

The spindle cells of SENs are positive for GFAP. GCs exhibit immunostaining for both GFAP and neuronal markers i.e. neurofilament, synaptophysin and MAP2.

### Subependymal giant cell astrocytoma (SEGA)

Subependymal giant cell astrocytomas (SEGAs) are the most common brain tumors occurring in about 5-10% of the TSC patients [18,24,30]. The pro-

gressive growth of enhancing lesions at the foramen of Monro in a TSC patient strongly suggests SEGAs. They generally exceed 1 cm in diameter but can reach a greater size. SEGAs usually extend into lateral ventricle and can obstruct the foramen of Monro and flow of CSF, causing hydrocephalus.

Macroscopically, SEGAs are gray to pinkish red in color, and sometimes develop massive haemorrhages and calcifications.

Microscopically, SEGAs consist of a mixed cell population with dysmorphic glial cells and GCs. The glial cells are polygonal, epithelioid, gemistocytic or spindle shaped with marked pleomorphism, dense eosinophilic cytoplasm, eccentric nuclei, prominent nucleoli, and nuclear inclusions (Fig. 6A,B). They are arranged in sheets, clusters (Fig. 6C), or pseudorosettes (Fig. 6D). The giant cells contain regular nuclei and abundant, eosinophilic cytoplasm (Fig. 6E). The characteristic feature of SEGAs is rich vascular stroma. SEGAs may contain numerous calcifications. Some mitotic figures (Fig. 6F) and foci of necrosis can be found. Inflammatory cell component, including mast cells and T lymphocytes can be detected [3].

### Immunohistochemistry

Immunoreactivity for GFAP (Fig. 7A), neurofilament (Fig. 7B), S-100, neuron-specific enolase, and synaptophysin proteins suggest that these tumors contain both glial and neuronal cell types and the name subependymal giant cell tumor (SEGT) is suggested. SEGAs do not stain with HMB45 in contrast to AML and LAM. There was minimal expression of p53 and high expression of bax protein. SEGAs show low labeling index of Ki67 about 2.9% [12].

Other brain neoplasms, for example glioma, ependymoma, schwannoma, medulloblastoma, and ganglioglioma can be found in TSC, but they are rather coincidental than syndromic associations. In two cases sacral chordomas were found to contain TSC gene mutations [22].

### Genetics

One third of patients have TSC history in their families, whereas two-thirds of all cases are caused by a “*de novo*” mutation or are the effect of the parental gonadal mosaicism.

The clinical signs of TSC are a result of mutations in either of two tumor suppressor genes: TSC1 (located on 9q34) or TSC2 (located on 16p13) [18]. About

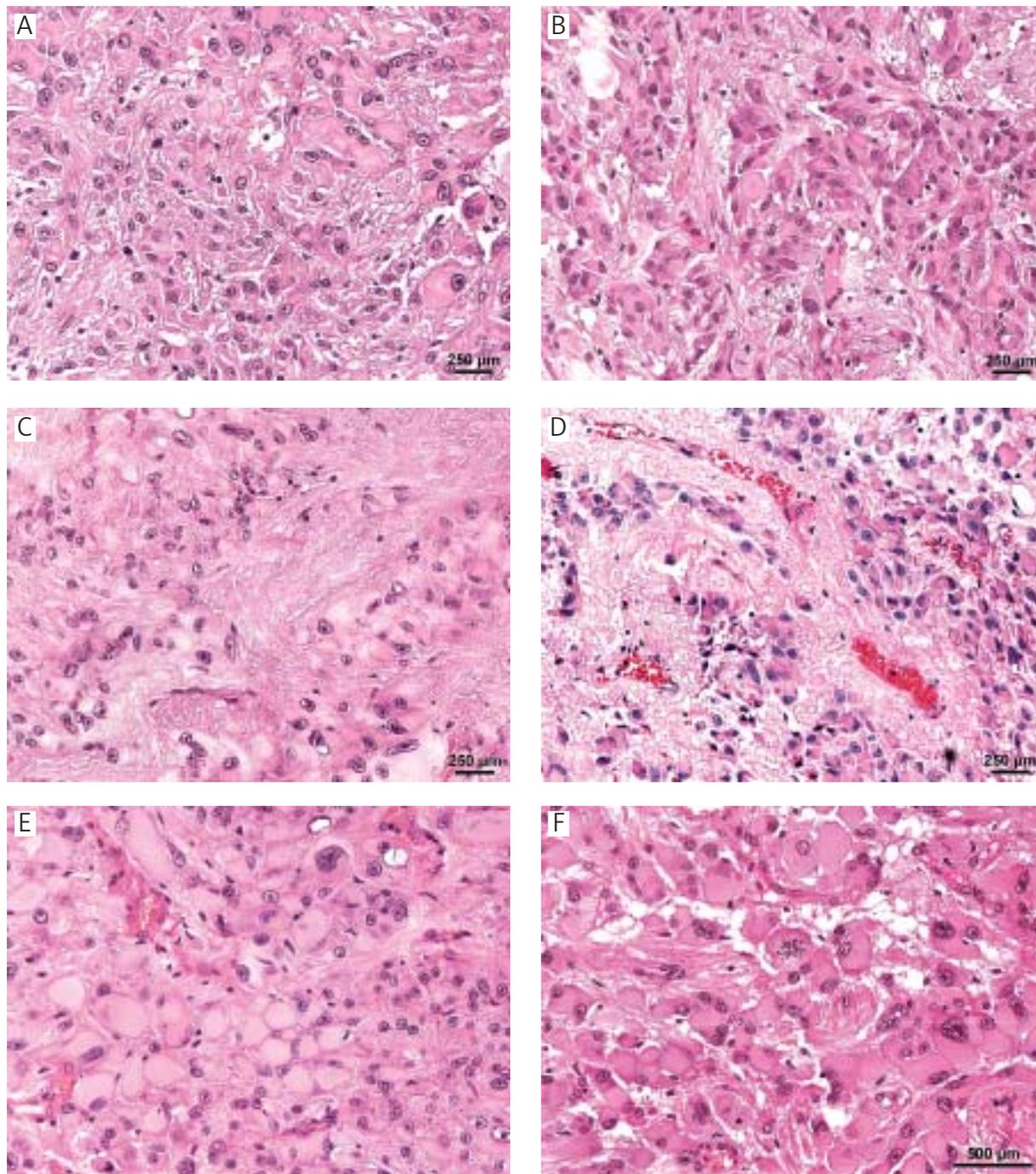
400 different mutations for both genes have been described [6,7,8,18]. Proteins encoded by TSC1 and TSC2 genes, hamartin and tuberin, respectively, form a heterodimer which suppresses the mammalian target of rapamycin (mTOR), a major cell growth and proliferation controller. mTOR is a serine-threonine kinase that receives input from many various signaling pathways to activate translation, and thus increasing cell proliferation, and growth. It is established that within the TSC complex, TSC1 stabilizes TSC2.

The heterodimer formed by tuberin and hamartin exerts GTPase (GAP) – activity towards a small G protein, Ras-homologue-enriched in brain (Rheb). Rheb is the direct target of the TSC1/TSC2 complex, leading to the inactivation of mTOR [5,13,18,19,25,31]. Rheb belongs to the family of G proteins, and, while being in its active GTP-bound state, stimulates mTOR, promoting protein synthesis and cell growth through phosphorylation of two downstream target proteins, S6K1 and 4E-BP1 (eukaryotic translation initiation factor 4E binding protein 1). S6K1 is a kinase that activates ribosomal subunit protein S6, leading to ribosome recruitment and protein translation. 4E-BP1 represses activity of eIF4E (eukaryotic translation initiation factor 4E) and, when phosphorylated by mTOR, releases eIF4E from its control (Fig. 8) [18].

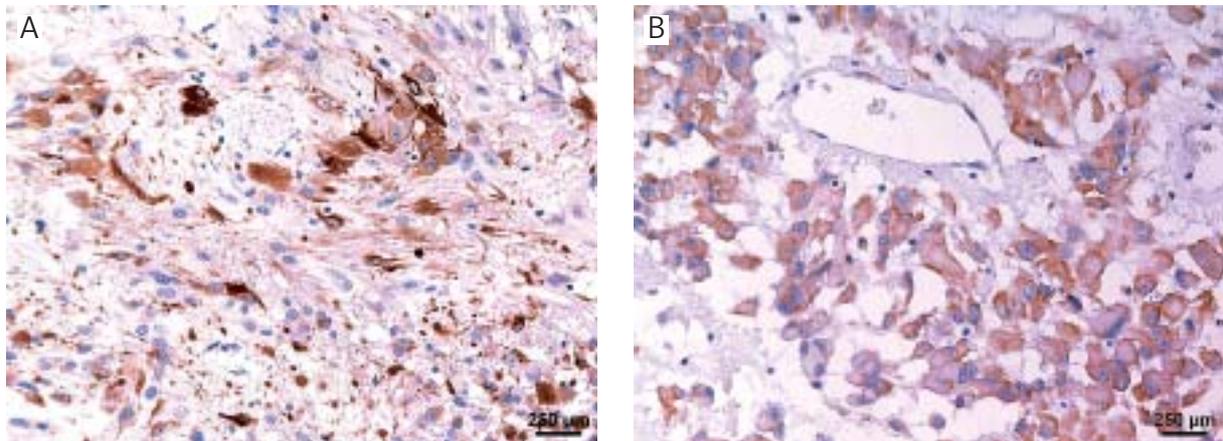
Tuberin and hamartin also bind independently of the complex to a variety of other proteins and regulate many important proteins (Fig. 9A,B). Tuberin inhibition results in proapoptotic protein Bad activation and increased apoptosis [10]. Cortical tubers in TSC were shown to have caspase-8 and Fas support cytokine activated. TUNEL examination showed ongoing cell death in cortical tubers associated with TSC. TSC proteins play also important role in autophagy [16].

TSC2-Rheb-mTOR pathway was also shown to control axon guidance and growth in central nervous system [18,19]. Additionally, the activation of mTOR leads to an increase of vascular endothelial growth factor expression, promoting tumor growth. However, the clinical significance of these interactions remains unclear.

Formation of tumors in TSC is believed to fulfill Knudson’s two-hit model of tumorigenesis. According to this model, explaining the tumor-suppressor gene function, the inactivation of both alleles of either TSC1 or TSC2 genes is required for tumor formation [18]. A germline mutation (spontaneous or inherited) inactivates one allele of one of these two genes. Somatic, second hit mutations affecting heterozy-



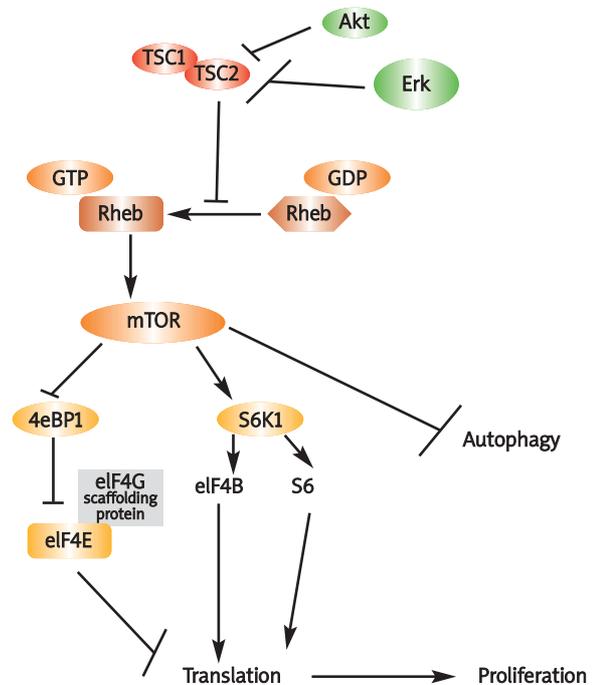
**Fig. 6.** Histopathology of SEGA. **A)** Polymorphic population of polygonal, epithelioid, gemistocytic or spindled cells with eosinophilic cytoplasm and eccentric nuclei, H&E; **B)** SEGA with large epithelioid, spindle and gemistocytic-like cells, H&E; **C)** Neoplastic cells arranged in clusters, H&E; **D)** Perivascular arrangement of cells similar to pseudorosettes, H&E; **E)** Giant cells containing regular nuclei and abundant, eosinophilic cytoplasm, H&E; **F)** Large eosinophilic cells with mitotic figure, H&E.



**Fig. 7.** Immunohistochemistry of SEGAs. **A)** Variable immunoreactivity for GFAP; **B)** Neoplastic cells immunopositive for neurofilament.

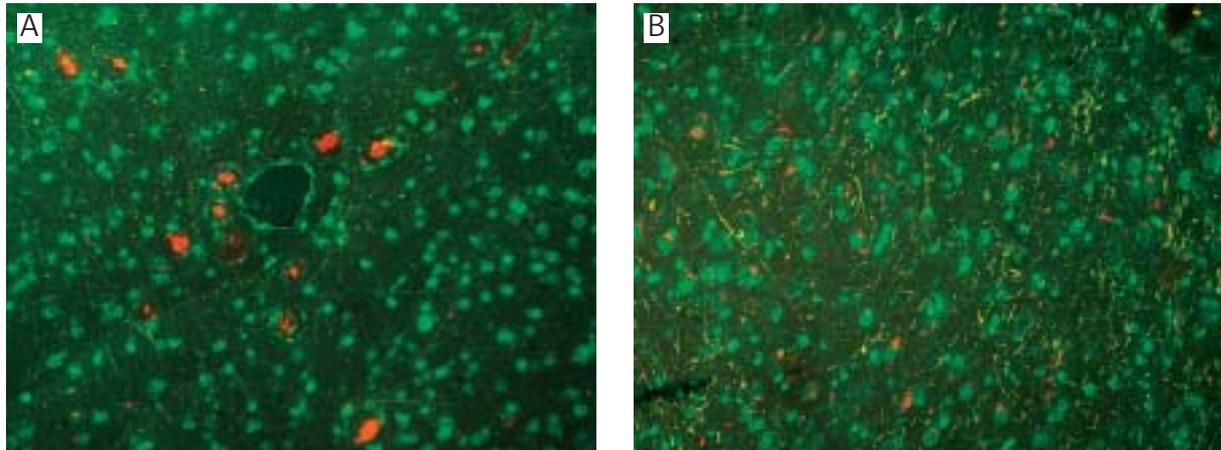
gous loci are referred to as loss of heterozygosity (LOH). In TSC, LOH is commonly found in kidney angiomyolipomas, but less frequently in cardiac rhabdomyomas, rarely in subependymal giant cell astrocytomas (SEGAs) and extremely rarely in cortical tubers [18]. Therefore, several hypotheses have been mooted to explain brain lesion pathogenesis in TSC. Many reports indicate that post-translational inactivation of TSC1/TSC2 complex arise through Akt kinase activation. Akt, a downstream effector of PI3K (phosphoinositide-3-OH kinase) is known to activate mTOR-dependent translation. The other mechanism of tumorigenesis in TSC is inactivation of the hamartin-tuberin complex after phosphorylation by various kinases, such as extracellular signal-regulated kinase (ERK), which was found in subependymal giant cell astrocytomas and cardiac rhabdomyomas [13]. TSC1-TSC2 complex stability may be also influenced by death associated protein kinase (DAPK). DAPK functions in a wide range of biological pathways, including TNF-regulated cell death, stress-induced apoptosis, and autophagy [10,16]. DAPK can mediate an inactivating phosphorylation of TSC2 and reduce its bioactivity after growth factor signaling. Consistent with these results, manipulation of DAPK by transfection or siRNA demonstrates that DAPK acts as a positive mediator of the mTORC1 translation pathway in response to growth factor stimulation.

Recently, gene expression profiling of SEGAs showed other genes possibly involved in these tumors development. Some genes implicated in tumorigenesis and nervous system development, like ANXA1, GPNMB, LTF, RND3, S100A11, SFRP4,



**Fig. 8.** Activation of Akt and Erk kinases leads to inactivating phosphorylation of TSC2. Disruption of TSC1/TSC2 complex releases mTOR inhibition and allows signaling from mTOR to S6K1 and 4E-BP1. As a consequence, protein translation is enhanced.

and NPTX1 are likely to be mTOR effector genes in SEGAs, as their expression was modulated by an mTOR inhibitor, rapamycin, in SEGAs-derived cells [13,29].



**Fig. 9.** Immunohistofluorescence images of cortical tuber samples **(A)** and SEGA **(B)** with primary anti-mTOR antibody. Green staining shows mTOR positive cells.

LOH has been reported in many TSC-related lesions, however it not found with the same incidence in all tissues and organs. LOH is more frequent in renal angiomyolipomas, less common in subependymal giant cell astrocytomas, and almost absent in cortical tubers [5,18]. Therefore, it is likely that haploinsufficiency may contribute to the development of lesions in some organs, most importantly in brain lesions [18].

### Conclusions and future direction

Rapamycin and its analogs are currently tested in several clinical trials in TSC. Various preclinical models showed that rapamycin treatment reduces TSC-related tumors, including brain, skin, and kidney tumors [23]. In clinical studies, oral rapamycin therapy lead to the regression of such hamartomas as SEGA, kidney AML [9,20,21] and LAM in TSC patients. Additionally, systemic rapamycin treatment has been found to be an antiepileptic medication in cases of epileptogenic cortical dysplasia and reduce seizures and cognitive defects in mouse models of TSC [23].

The understanding of TSC pathogenesis may provide useful data for neuroscientists working on brain tumors, epilepsy and neuronal migration disorders. It is yet known, that normal function of TSC1 and TSC2 genes and proteins is very important for brain development, because it regulates cortical lamination, axon outgrowth, and cell size. However, still many issues require further studies. Firstly, understanding how loss of TSC1 or TSC2 influences cell migration may explain the origin of disordered lamination in

tubers and cortical dysplasia. Secondly, defining mechanisms that are responsible for conversion from SEN to SEGA and then the uncontrolled growth of SEGAs could yield new strategies for brain tumors. Finally, defining the role of TSC1 or TSC2 in infantile spasms generation and intractable epilepsy would provide novel approaches to epilepsy management.

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