

Expression of tuberin and hamartin in tuberous sclerosis complex-associated and sporadic cortical dysplasia of Taylor's balloon cell type

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Abstract

Focal cortical dysplasia (FCD) type IIB is a malformation of cortical development characterized by presence of balloon cells. These cells share phenotypic features of giant cells found in tuberous sclerosis complex (TSC), but the relationship between FCD type IIB and TSC is not well established. TSC is an autosomal dominant disorder caused by mutation in either of two genes: TSC1, encoding hamartin, and TSC2, encoding tuberin. Both proteins form a complex inhibiting mTOR signalling pathway and thus regulate cell size and proliferation.

In this study, tuberin and hamartin expression was evaluated under a confocal microscope in six cases of Taylor's balloon cell type FCD. Three patients met the clinical criteria for TSC. In three other patients, TSC was excluded based on a panel of clinical and radiological examinations. Additionally, two cases of FCD type I and 3 samples of normal brain tissue were used as a reference group.

We found loss of tuberin and hamartin expression in FCD type IIB lesions from patients with TSC. In sporadic FCD type IIB cases, only a few tuberin and hamartin positive cells were detected in the white-grey matter junction and in deeper parts of the white matter. Cortical balloon cells showed loss of both tuberin and hamartin. In contrast, the expression of tuberin and hamartin in FCD type I samples was strong, similarly to normal brain tissue.

In conclusion, loss of TSC1 and TSC2 products expression in balloon cells of both cortical dysplasia type IIB in TSC-related and sporadic patients suggests that FCD type IIB may represent the focal form of TSC.

Key words: balloon cells, TSC, FCD type IIB, tuberin, hamartin.

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Introduction

Malformations due to abnormal cortical development are now recognized as one of the major aetiologies causing intractable epilepsy in children [7]. A recent classification divides neuronal migration disorders into mild cortical dysplasias (MCDs) and focal cortical dysplasias (FCDs), with respect to cytoarchitectural criteria [26]. Cortical dysplasias with isolated architectural abnormalities such as dyslamination are classified as FCD type I. FCD type II is restricted to specimens with architectural abnormalities and prominent dysmorphic cellular components. FCD type IIB, so-called Taylor's type, is characterized by presence of balloon cells in the deep cortex and white matter [5,26]. Balloon cells are increased in size and have an eosinophilic cytoplasm and an eccentric, pleomorphic nucleus. The pathogenesis of balloon cells is not clear. It was shown that balloon cells demonstrate a mixture of immunohistochemical markers of both neuronal and glial origin [8,14,32].

Balloon cells are also a hallmark of tuberous sclerosis complex (TSC) brain pathology, namely cortical tubers and subependymal giant cell astrocytomas (SEGAs). TSC is an autosomal dominant genetic disorder seen in about 1 : 6000 live births, that is characterized by formation of tumour-like growths (hamartomas) in the brain, skin, kidneys, heart and other organs [4,23]. TSC occurs due to germline or mosaic mutation in either of two genes: TSC1 or TSC2 [6]. The protein product of TSC1 gene, hamartin, consists of 1164 amino acids with a calculated mass of 130 kDa [19]. TSC2 gene encodes tuberin, a protein of 1784 amino acids and 198 kDa [13]. Both proteins are widely expressed in human tissues, including brain, and they appear to co-localize in most cells [10,11,27,28]. The mechanisms through which TSC1 and TSC2 control cellular growth and proliferation have been partially elucidated, with light being shed into the dynamic interaction between hamartin and tuberin, forming a tumour suppressor heterodimer. This complex inhibits the mammalian target of rapamycin (mTOR). mTOR is a key regulator in the signalling pathway of cell proliferation and organ size, phosphorylating 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. It was

recently demonstrated that Rheb (Ras homologue enriched in brain) is the direct target of TSC1/TSC2 complex, leading to the inactivation of mTOR. Rheb belongs to the family of G signal transducing proteins. The TSC1/TSC2 heterodimer switches Rheb from an active to an inactive state leading to cell growth arrest [4,15].

Elimination of tuberin or hamartin from cells leads to activation of S6K1 and 4E-BP1 kinases, and a resulting cell growth advantage. In most TSC-related hamartomas both tuberin and hamartin expression is reduced or, more often, absent [4,16,17,20-22].

Cortical tubers in TSC share virtually all neuropathological features of classical Taylor-type cortical dysplasia type IIB [24,26]. However, the molecular mechanisms underlying FCD type IIB are not clear. It is also not known whether FCD type IIB found in TSC patients differs from sporadic cases. Our preliminary report suggested that balloon cells in TSC and FCD type IIB may share the same origin [14]. We postulated that FCD type IIB may in fact present the focal form of TSC. To elucidate further this issue, we studied tuberin and hamartin expression in TSC-related as well as sporadic FCD type IIB.

Material and Methods

The study was approved by the local Ethics Committee, The Children's Memorial Health Institute, Warsaw. The 6 cases included in this study were from the Department of Pathology, The Children's Memorial Health Institute, Warsaw. Patients underwent resection of FCD for medically intractable epilepsy. In each case, the histological diagnosis of Taylor's balloon cell type FCD was confirmed by standard methods. Three patients met the clinical criteria for TSC. In two of them, genetic examination was performed and TSC2 mutation was found in both cases. In 3 other patients, TSC was not confirmed on the basis of negative skin examination and kidney, liver and heart USG as well as the neuroimaging study revealing FCD as the sole pathology. All samples were fixed overnight in 4% formalin and routinely processed into paraffin.

Two paraffin embedded blocks of cases with FCD type I and 3 samples of normal brain tissue, gained from the same Department of Pathology, were used as a reference group.

Sections of 10 µm were made by means of Reichert Jung Microtome. The sections were mounted on slides

covered with NovoBond (Novocastra, England), air dried for 24 hours and deparaffinized through xylene and graded alcohols to PBS (phosphate-buffered saline; SIGMA). To enable antibody binding, the high-temperature antigen unmasking method using citrate buffer was applied. The unspecific binding sites were blocked in 10% BSA (bovine serum albumin) and 10% goat serum (both SIGMA). The sections were then treated simultaneously with anti-tuberin (Novocastra; 1:25 dilution) and anti-hamartin (Santa Cruz, USA; 1:50 dilution) primary antibodies. We used anti-tuberin mouse monoclonal antibody recognizing the 240 amino acid N-terminal region of human tuberin and anti-hamartin rabbit polyclonal antibody directed against the 300 amino acid N-terminal region of human hamartin. The specimens were kept in a humid chamber, 37°C, for 1 hour. After triple rinsing in PBS, the mixture of secondary antibodies was applied. These antibodies were goat anti-mouse IgG conjugated with AlexaFluor 488 and goat anti-rabbit IgG conjugated with AlexaFluor 568 (both Molecular Probes, USA). Staining was performed in a moist chamber, room temperature, for 2 hours. Then, after triple rinsing in PBS, the slides were covered with VectaShield (Vector, USA) and coverslipped. The specimens were examined under an Olympus Fluoview confocal microscope (Olympus, Japan). Merged pictures of both excitation wavelengths were made and analyzed.

Results

Histopathologically, the surgical specimens with FCD type I exhibited abnormal microcolumnar arrangement of cortical neurons and presence of ectopic neurons in white matter (Fig. 1). The cases diagnosed as FCD type IIB showed advanced disorganization of laminar architecture accompanied by numerous dysplastic neurons and typical balloon cells (Fig. 2). The balloon cells displayed enlarged, round cell bodies with glassy, eosinophilic cytoplasm and eccentrically located nucleus (Fig. 3). Multi-nucleated cells were seen and sometimes exhibited intranuclear pseudoinclusions.

In all control normal brains, as well as in FCD type I samples, strong tuberin and hamartin expression was documented in the majority of neuronal cells and other cellular elements. Both these proteins were often co-localized. Tuberin and hamartin immunoreactive cells were found in the cortex and in white matter (Fig. 4).

In FCD type IIB specimens from patients with TSC, there was no expression of either tuberin or hamartin. Neither balloon cells nor other cellular elements of the specimen showed immunoreactivity for tuberin and hamartin (Fig. 5). In sporadic FCD type IIB cases, a few tuberin and hamartin positive cells were detected only at the white-grey matter junction and in deeper parts of the white matter, whereas cortical balloon cells showed distinct loss of both tuberin and hamartin (Fig. 6).

Interestingly, the dysplastic cortex in non-TSC patients was at times bordered by normally appearing brain tissue that was immunopositive for both tuberin and hamartin. However, in TSC patients, normal-appearing brain tissue surrounding the dysplastic region of the cortex was negative for hamartin and tuberin.

Discussion

In the present study we used confocal microscopy to detect the expression and co-expression of both TSC-related proteins, hamartin and tuberin, in TSC-related and sporadic focal cortical dysplasia type IIB. We found strong tuberin and hamartin expression in normal brain tissue and in FCD type I. Tuberin and hamartin positive cells were seen both in the cortex and in the white matter. In FCD type IIB, both sporadic and TSC-related, tuberin and hamartin expression was markedly reduced or even absent. Cortical balloon cells were negative for tuberin and hamartin in all FCD type IIB samples.

Tuberin and hamartin are widely expressed in most human tissues and they usually share their localization; however, some differences have been noticed [11,13,29]. Tuberin demonstrates a diffuse cytoplasmic expression pattern, while hamartin distribution is more punctate. In the brain, hamartin is predominantly found along neuronal and astrocytic processes, whereas in the perinuclear region of Purkinje cells tuberin is distributed solely [11]. Tuberin and hamartin are tumour suppressor proteins which have been found mutated in TSC. However, investigations on both TSC-related proteins in TSC-related hamartomas are limited in the available literature. Moreover, while immunohistochemical studies in TSC-related extra-neuronal tumours showed loss of both TSC1 and TSC2 products expression, the data obtained from brain lesions were conflicting. Henske et al. [12] found loss of tuberin in a few analyzed SEGAs. In SEGAs and cortical tubers,

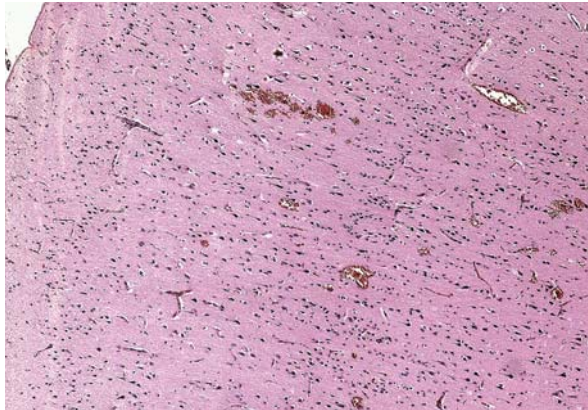


Fig. 1. Neuropathology of FCD type I. Distinct microcolumnar arrangement of cortical neurons. H&E. Original magn. $\times 40$

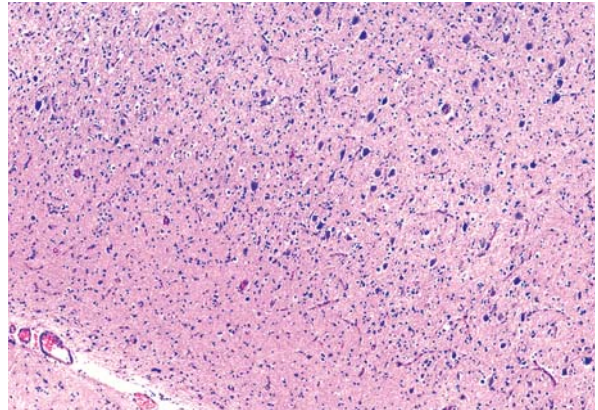


Fig. 2. Disorganization of cortical laminar architecture and dysplastic neurons in FCD type IIB. H&E. Original magn. $\times 40$

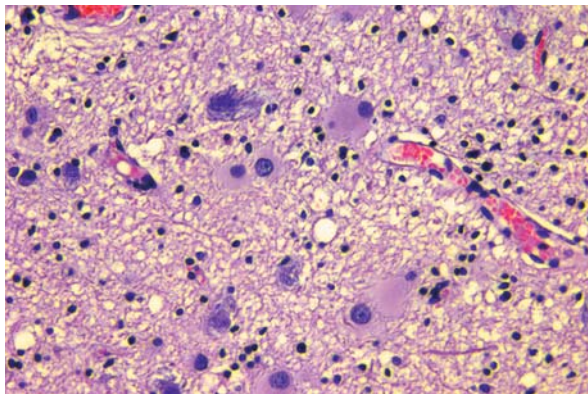


Fig. 3. FCD type IIB with balloon cells exhibiting abundant, pale, ground-glass cytoplasm and multiple eccentrically placed nuclei. H&E. Original magn. $\times 200$

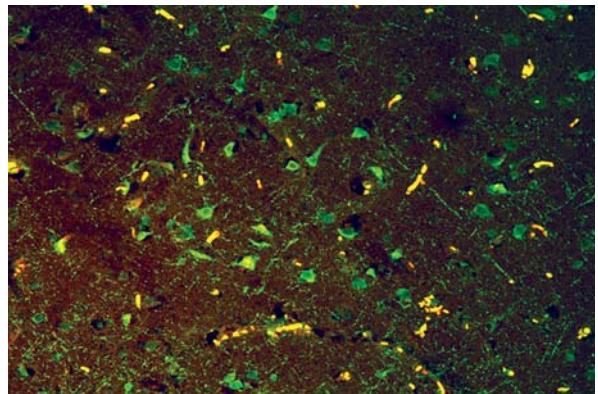


Fig. 4. Co-localisation of tuberlin (green) and hamartin (red) expression in control brain tissue (confocal microscope). Red and green channel merged (double stained cells are yellow). Original magn. $\times 200$

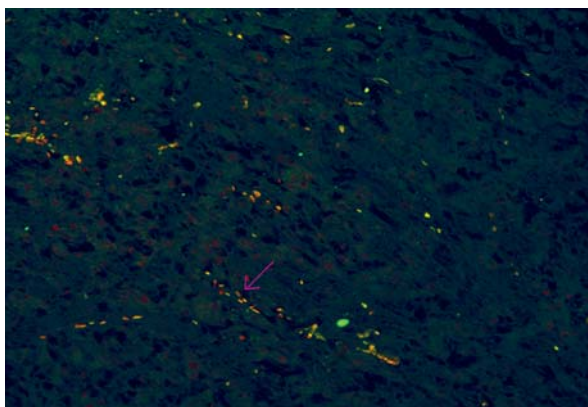


Fig. 5. Confocal microscopy of TSC-related FCD type IIB, double (red and green channel merged) stained for hamartin and tuberlin. An arrow indicates autofluorescent red blood cells. Original magn. $\times 200$

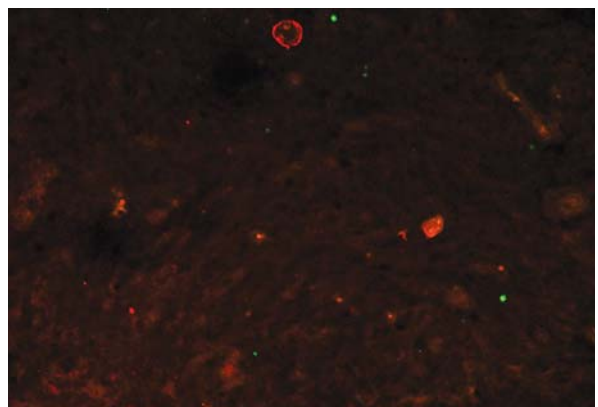


Fig. 6. Confocal microscopy of sporadic FCD type IIB, double (red and green channel merged) stained for hamartin and tuberlin. Original magn. $\times 200$

Mizuguchi [20,21] found loss of both proteins expression using Western blot and, surprisingly, reduced but present labelling in the same tumour, when using immunohistochemistry. Some authors found loss of tuberlin immunostaining in spindle and epithelioid but not giant cells in SEGA [1,21,28]. Our previous study revealed loss of both tuberlin and hamartin expression in ten SEGAs samples. Interestingly, loss of both tuberlin and hamartin expression did not depend on TSC1 or TSC2 mutation [16].

In cortical dysplasia, the data concerning tuberlin and hamartin are scant and even more confusing. Becker et al. studied the alterations in TSC1 and TSC2 genes in a cohort of patients with focal cortical dysplasia [3]. By means of microdissection and laser-assisted isolation they obtained DNA from balloon cells and dysplastic neurons. Sequence alterations resulting in amino acid exchange in the TSC1 gene were increased in FCD samples when compared to normal brain tissue. Moreover, they found loss of heterozygosity at the TSC1 gene locus in nearly half of FCD samples. Ljungberg et al. [18] reported similar activation of the mTOR pathway in cytomegalic neurons in cortical dysplasia and TSC lesions. These findings further support our previous work [14] describing the molecular similarities between balloon cells in TSC and cortical dysplasia. On the other hand, Baybis et al. [2] found reduced tuberlin mRNA levels in balloon cells obtained from TSC cortical tubers but not in giant cells obtained from cortical dysplasia. They used cDNA array analysis to study mRNA from single microdissected cells obtained from TSC cortical tubers and cortical dysplasia. Several differences in gene expression were observed between tubers and dysplasia samples; thus the authors suggested that these lesions differ in pathogenesis.

The occurrence of hamartomas in tuberous sclerosis is commonly ascribed for the loss of heterozygosity (LOH) phenomenon. In contrast to heart, kidney and lung tumours, brain lesions in TSC rarely present LOH for TSC1 or TSC [4,15]. Nevertheless, tuberlin and hamartin are usually lost or markedly reduced in these lesions. Moreover, regardless of TSC1 or TSC2 mutation found in a particular patient, loss of both tuberlin and hamartin proteins was reported in SEGAs [16]. In the present study, we also found loss of both proteins in TSC-related and sporadic FCD type IIB. Loss of both proteins seems to be a unique feature of TSC lesions as in some sporadic

astrocytomas and gangliogliomas, tuberlin was reduced, but no disturbances in hamartin expression were found [27,31]. These findings suggest separable roles of tuberlin and hamartin in sporadic tumour development.

In conclusion, our results confirmed the suggestion that FCD type IIB with balloon cells and TSC brain lesions with classical Taylor-type cortical dysplasia type IIB might represent the same entity or that FCD with balloon cells might simply be a solitary form of TSC [30]. This similar pattern of TSC1 and TSC2 products expression indicates that TSC brain lesions and FCD type IIB may share common pathogenesis. The problem whether FCD type IIB is truly a focal form of tuberous sclerosis requires further studies using more samples and genetic analyses. Recently, some animal as well as clinical trials of rapamycin in brain lesions associated with tuberous sclerosis complex have been reported [9]. The use of this immunosuppressant acting by inhibition of mTOR seems to be promising in TSC. If FCD type IIB is derived by the same pathomechanism, such treatment might also be reasonable in cortical dysplasia associated with drug-resistant epilepsy.

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