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Identification and immunophenotype of abnormal cells present in focal cortical dysplasia type IIb

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Abstract

Background: Focal cortical dysplasias (FCDs) are malformations of cortical development that present cortical dyslamination and abnormal cell morphology and are frequently associated with refractory epilepsy. FCD type IIb presents dysmorphic neurons (DNs) and balloon cells (BCs), which are the hallmarks of this dysplasia. Moreover, hypertrophic neurons (HyNs) may be present in FCD types I, II and III. The objective of this study was to perform a detailed morphology and immunophenotype study of BCs, DNs, and HyNs in a cohort of FCD IIb patients.

Methods: Cortices resected as a treatment for refractory epilepsy from 18 cases of FCD type IIb were analysed using Bielschowsky method and haematoxylin and eosin as routine stains. Immunophenotype was performed using specific antibodies to detect epitopes differentially expressed by abnormal cells.

Results: All cases showed cortical dyslamination, BCs, DNs, and HyNs. No cell layer or column could be identified, except for cortical layer I. Lesions predominated in the frontal cortex (11 cases). DNs were large neurons and presented a clumped and or displaced Nissl substance towards the cell membrane, and a cytoplasm accumulation of neurofilament that displaced the nucleus to the cell periphery, as shown by Bielschowsky staining and immunohistochemistry. HyNs were as large as DNs, but without alterations of Nissl substance or dense neurofilament accumulation, with a central nucleus. BCs were identified as large, oval-shaped and pale eosinophilic cells, which lacked the Nissl substance, and presented an eccentric nucleus. BCs and DNs expressed epitopes of both undifferentiated and mature cells, detected using antibodies against nestin, vimentin, class III β-tubulin, pan-neuronal filaments, neurofilament proteins, β-tubulin and NeuN. Only BCs expressed GFAP.

Conclusion: FCDs present with disorganization of the cerebral cortex architecture, abnormal cell morphology, are frequently associated with refractory epilepsy, and their post-surgical prognosis depends on the type of FCD. The diagnosis of focal cortical dysplasia in a surgical specimen relies on the identification of the abnormal cells present in a dysplastic cortex specimen. The current report contributes to the identification of balloon cells, dysmorphic and hypertrophic neurons in the context of focal cortical dysplasia type IIb.

Keywords: Focal cortical dysplasia type IIb, Taylor's focal cortical dysplasia, Dysmorphic neuron, Hypertrophic neuron, Balloon cell, Tuberous sclerosis complex, Hemimegalencephaly

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Introduction

Malformations of cortical development (MCDs) can result from disturbances of one or several processes that lead to cerebral cortex formation, which include proliferation, migration, differentiation, synaptogenesis, and apoptosis of neural cells (Palmini 2000; Desikan and Barkovich 2016). MCDs include a heterogeneous group of disorders that can affect broad regions of the cerebral cortex, as in hemimegalencephaly (HME), or may be restricted to focal areas such as tubers in the tuberous sclerosis complex (TSC) or Taylor's type focal cortical dysplasia (FCD type IIb) (Crino et al. 2002). Neuropathology of HME, TSC and Taylor's FCD share a disruption of the cerebral cortex structure, aberrant cell morphologies and underlying abnormalities in the white matter. These aberrant cells include balloon cells (BCs), dysmorphic (DNs) and hypertrophic (HyNs) neurons (Crino et al. 2002; Palmini et al. 2004; Aronica and Mühlebner 2017).

Focal cortical dysplasia was first defined by (Taylor et al. 1971) who described, in the neocortex of patients with drug-resistant epilepsy, a localized disruption of the normal cortical lamination with large bizarre neurons and BCs. (Palmini et al. 2004) classified FCDs in types I and II. Types Ia and Ib present architectural disturbances of cortical lamination, and type Ib additionally presented HyNs. Types IIa and IIb both present DNs, but only type IIb presents BCs. DNs and BCs are the hallmark of FCD type IIb, or Taylor's FCD (Tassi et al., 2002; Palmini et al. 2004; Blümcke et al. 2011). An ad hoc ILAE Task Force proposed a new classification system. ILAE Task Force reviewed the available literature on clinical presentation, imaging findings, and histopathologic features of distinct FCD variants, and proposed a more refined clinico-pathologic classification system, added an FCD Ic subtype, and added FCD type III with four subtypes, but maintained (Palmini et al. 2004) types IIa and IIb categories (Blümcke et al. 2011). By analogy to the WHO recommendation for the classification of tumors based on integrated clinico-pathological and genetic basis, the ILAE FCD classification was recently reviewed (Najm et al. 2018). It added the possible use of markers of mTOR pathway mutations to identify FCD type II, included an FCD type IIb variant, the bottom-of-sulcus FCD (Harvey et al. 2015), and raised the question whether FCD IIa, IIb and variant FCD type II are a spectrum of FCD type II, among other considerations.

Taylor et al. (1971) thought that the condition they described appeared to be a congenital malformation that resembled tuberous sclerosis, but suggested that the relation to TSC appeared to be remote. Indeed, cortical specimens of FCD type IIb are sometimes misinterpreted as a somewhat forme fruste of TSC. However, recent findings indicated that mutations of the mTOR pathway associated with TSC also occur in FCD types IIa and IIb (Najm et al. 2018; Curatolo et al. 2018). In addition to the similarity between FCD IIb and TSC, another difficulty regarding FCD type IIb diagnosis is the identification of HyNs and DNs, because it may not be straightforward. Also, the histopathologic distinction between FCD type IIa and type IIb may be problematic, if non-representative or small surgical specimens are submitted for microscopical inspection (Blümcke et al. 2011). Focal cortical dysplasia is a common malformation of cortical development, mostly in children under 3 years of age, and in adults with refractory epilepsy undergoing surgery for seizure control. Therefore, a detailed morphology and immunophenotype study of the cortical cytoarchitecture and of the abnormal cells that are the hallmark of FCD type IIb, and the criteria for the identification of BCs, DNs, and HyNs, of using bonafide cases, was performed and discussed.

Materials and methods

Patients

Patients were evaluated at the Ribeirão Preto Epilepsy Surgery Program (CIREP) at Ribeirão Preto Medical School, University of São Paulo, SP, Brazil, at the Porto Alegre Epilepsy Surgery Program, Porto Alegre, Brazil, and at the Clinical Epileptology and Experimental Neurophysiology Uni, Fondazione IRCCS Istituto Neurologico "C. Besta", using standardized protocols. The Ethics Committees of our Institutions, Hospital das Clínicas, Ribeirão Preto Medical School, University of São Paulo, process 9370/2003 e Comissão de Ética em Pesquisa da Universidade Federal do Triângulo Mineiro, process No. 1229, evaluated and approved the use of biopsies and the publication of the study results, on the basis of the informed, written consent given by the patients. Cases for this study were selected from patients with drug-resistant epilepsy who underwent surgical resection of the epileptogenic region for treatment. Patient evaluation included a detailed history and neurologic examination, interictal scalp EEG, interictal/ictal video-EEG monitoring, a neuropsychological test battery, and intra-operative cortical recording. Video-EEG monitoring was performed on all patients. Neuroimaging included high-resolution magnetic resonance imaging (MRI), and ictal and interictal single-photon emission computerized tomography (SPECT) scans. Identified epileptogenic regions were resected.

Tissue processing and immunohistochemistry

Cortical specimens were fixed in 10% (weight/volume) 0.1 M phosphate-buffered formalin, pH 7.5, and paraffin embedded. Cells were identified on the basis of their morphologies on H&E, Nissl, Bielschowsky's staining and immunohistochemistry assay. Epitopes studied were: glial fibrillary acidic protein (GFAP), vimentin, nestin,

β-tubulin, neuronal class III β-tubulin, NeuN, neurofilament protein and pan-neuronal filaments (antibody SMI 311) and microtubule-associated protein-2 (MAP2) (Table 1).

The detection of antibodies in 4–8 μm tissue sections was performed according to Martins et al. 1999, 2011 (Martins et al. 1999; Martins et al. 2011), antigen retrieval was done using 50 mM Tris-HCl buffer, pH 9.5, and detection was carried out using the ABC technique (Vectastain Elite ABC kit) and diaminobenzidine (Pierce, cat. 34,001) as chromogen. Sections were then incubated overnight with antibodies directed against the above epitopes, at the indicated dilutions in blocking buffer (Table 1). After each incubation, sections were washed with Triton buffer [50 mM sodium phosphate buffer, pH 7.5, containing 0.9% (weight/volume) NaCl and 0.03% Triton X-100 (volume/volume) (USB, cat. 22,682)]. Endogenous biotin was blocked using the Biotin Blocking System (Dako, cat. 0590). Sections were then incubated for 1 h with biotinylated swine anti-rabbit IgG, or rabbit anti-mouse IgG diluted in blocking buffer (Triton buffer containing 15% (volume/volume) normal goat serum and 3% (weight/volume) bovine serum albumin (Sigma, cat. 113 k0643). All operations were carried out at room temperature. Primary antibodies were omitted in the control sections.

Results

Clinical findings

We studied the cortical tissue from 18 patients presenting FCD type IIb (8 males), which underwent surgery for the treatment of refractory epilepsy. Patient mean age at surgery was 18.2 ± 13.9 (range < 1–41) years (Table 2). The epileptogenic lesions in FCD IIb patients were distributed over diverse cortical regions but predominated in the frontal cortex (11 cases). Multilobar lesions were seen in two cases. Four patients had a family history of epilepsy. Three patients were operated twice, and one died after surgery.

Histopathologic diagnosis

Patients presenting refractory epilepsy whose clinical, imaging and electroencephalographic studies suggested a cortical lesion compatible with FCD type IIb (Table 2) underwent surgical treatment. The resected cortices were studied using routine stains (H&E, cresyl violet and silver impregnation by the Bielschowsky's method), and a panel of specific antibodies (Table 1).

H&E staining of a cortical section (Fig. 1a) from a patient whose frontal cortex was resected for treatment of refractory epilepsy showed a disruption of the cortical organization, which included both laminar and columnar architecture organization. Dyslamination was severe, and

Table 1 Antibodies used in the present immunohistochemistry assays

Antibody	Host	[a]Dilution	Source/catalog	Immunogen	Description
Primary antibodies					
Anti-glial fibrillary acidic protein	Rabbit	1:500	Dako/Z0334	Bovine glial fibrillary acidic protein (GFAP)	Astrocyte marker
Anti-vimentin, clone V9	Mouse	1:500	Dako/M0725	Pig vimentin	Mesenchymal cell marker
Anti-nestin, clone rat-401	Mouse	1:100	Chemicon/MAB353	Rat nestin	Marker of intermediate filament protein (class type VI) expressed during development
Anti-β-tubulin, clone N357	Mouse	1:200	Amersham/N357	β-tubulin	Microtubules marker
Anti-tubulin, neuronal class III β-tubulin, clone TU-20	Mouse	1:50	Millipore/MAB1637	Synthetic peptide corresponding to amino acids 443–450 of human class III beta-tubulin conjugated to KLH	Reacts with the C-terminus of the neuron specific β-III isoform of tubulin
Anti-NeuN, clone A60	Mouse	1:200	Chemicon/MAB377	Purified cell nuclei from mouse brain	Marker of neuron-specific nuclear protein
Anti-neurofilament protein, clone 2F11	Mouse	1:100	Dako/M0762	Neurofilament M isolated from normal adult human brain	Neuron marker
Antibody against pan-neuronal neurofilament	Mouse	1:800	Covance/SMI-311	Neuron intermediate filaments	Neuron marker
Anti-microtubule associated protein-2, clone AP20	Mouse	1:200	Millipore/MAB3418	Bovine brain microtubule protein-2	Marker of neuronal cell bodies and dendrites
Secondary antibodies					
Anti-mouse IgG	Rabbit	1:100	Dako/E0354	Rabbit IgG conjugate biotin	–
Anti-rabbit IgG	Pig	1:100	Dako/E0353	Pig IgG conjugate biotin	–

[a]All antibodies were diluted in 50 mM sodium phosphate buffer, pH 7.5, containing 0.09% (weight/volume) NaCl, 0.03% (volume / volume) Triton X-100, 3% (weight/volume) bovine serum albumin and 15% (volume / volume) goat serum

Table 2 Clinical features of patients with FCD IIb in the present cohort

Case number	Sex	Age at surgery (year)	Age of epilepsy onset (year)	Approximate seizure frequency (per month)	Duration of epilepsy (years)	Epileptogenic region
1	M	6	< 1	400	6	R-Fr
2	F	19	1	180	18	R-T
3	F	34	3	30	31	R-T
4	F	41	3	30	38	R-Fr
5	M	5	< 1	150	5	R-TPO
6	F	2	< 1	150	2	R-T
7	F	5	< 1	150	5	R-TO
8	F	< 1	< 1	150	0.75	L-Fr
9	M	3	1	150	2	L-Fr
10	F	16	8	90	8	Fr
11	M	22	4	30	18	L-Fr
12	F	21	4	30	17	Fr
13	F	12	< 1	4	12.5	R-O
14	F	9	6	120	9	L-Fr
15	M	20	7	30	13	Fr
16	M	35	2	30	33	L-Fr
17	M	40	19	4	21	R-T
18	M	36	19	30	17	L-Fr

M male, *F* female, *R* right, *L* left, *Fr*, *T*, *P*, *O* are for frontal, temporal, parietal and occipital lobes, respectively

no layer or column was observed, except for layer I (case 11, Fig. 1b). However, even layer I in case 10 was invaded by large cells (Fig. 1a and c). Large and abnormal cells observed in this study included BCs (arrows in Fig. 1a, c, d, and g) and DNs (Fig. 1e), which are the hallmarks of FCD IIb. Other frequent findings observed here included misdirection of the apical dendrites, many of which were not oriented towards the pial surface (PS) (Fig. 1b, f), HyNs (open arrowhead in Fig. 1a, i), blurring of the gray-white matter transition (Fig. 1g), and heterotopic neurons in the white matter (Fig. 1h). Increased glial numbers and reactivity were a frequent finding, conspicuously shown here in the vicinity of HyNs (Fig. 1i). All 18 patients studied here presented cortical dysplasia, BCs, DNs, and HyNs. Normal appearing or less dysplastic cortical areas were usually observed at the border of the dysplastic cortex.

Characterization of balloon cells, dysmorphic and hypertrophic neurons

Routine stains currently used in pathology, e.g., H&E, cresyl violet and silver impregnation by the Bielschowsky's method, permitted the first level of diagnosis of abnormal cells present in FCD type IIb (Fig. 2), such as BCs, DNs, and HyNs. BCs (Fig. 2a, b, c) were identified by their oval, large cell body, a large, pleomorphic and eccentric nucleus, and a prominent nucleolus (Fig. 2b, cresyl violet). H&E staining of BCs showed a homogeneous, glassy and eosinophilic cytoplasm without an observable Nissl substance (Fig. 2a). BCs stained by the Bielschowsky's method showed a characteristic homogeneous, golden-brown cytoplasm (Fig. 2c). BCs were observed at all cortical depths, but a preferred localization was the white matter and the grey-white matter transition. DNs (Fig. 2d, e, f) were identified by their large cell bodies and nuclei diameters, as compared to pyramidal neurons of layer V. DNs stained by H&E (Figs. 1e and 2d, e) showed a Nissl substance clumped, or clumped displaced towards the cell membrane. DNs presented eccentric nuclei due to filament accumulation (Fig. 2f, silver impregnation). HyNs were identified by their centrally positioned nuclei and large cell bodies (Fig. 2g-j). The cytoplasm of HyNs was usually homogeneous, without an observable Nissl substance (Fig. 2g). They presented an overall morphology similar to that of pyramidal neurons (Fig. 2h, j) or interneurons (Fig. 2g). HyNs expressed, but not overexpressed, neurofilament protein (Fig. 2i) and pan-neuronal filaments (Fig. 2j), which are markers of mature neurons.

Balloon cells, dysmorphic and hypertrophic neurons expressed markers of both mature and undifferentiated cells

The Immunophenotyping of BCs, DNs, and HyNs was carried out using a panel of specific antibodies (Table 1). BCs expressed nestin (Fig. 3a) and vimentin (Fig. 3b;

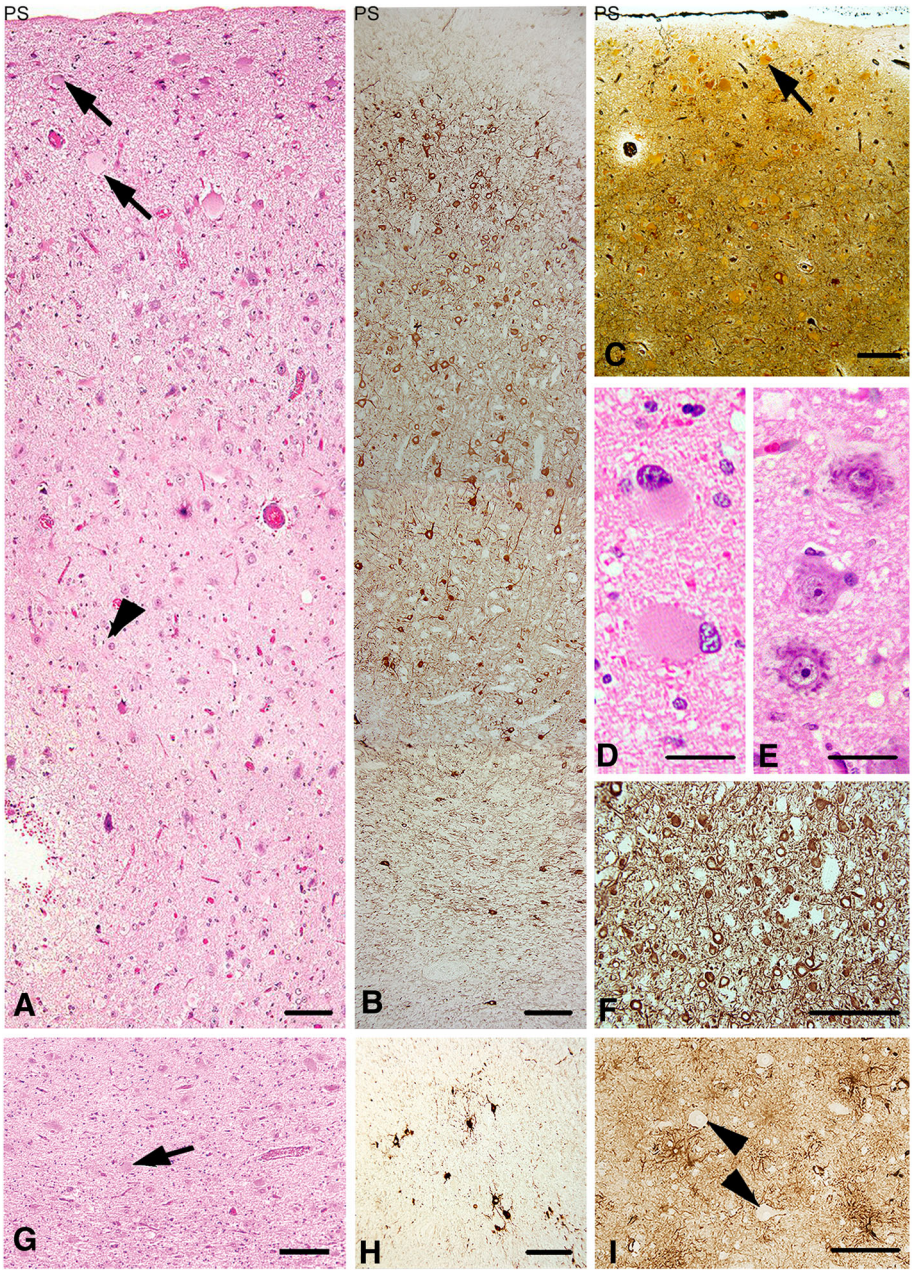

Fig. 1 Histopathologic findings in focal cortical dysplasia type IIb. Sections of frontal cortices resected from epileptic patients showed strongly dysplastic regions, which were characterized by both laminar and columnar architecture disorganization (**a, b, c**). Layer I was observed in most cases (**b**), but in others layer I was populated by abnormal cells (**a, c**). Both BCs (arrows in **a, c, d**, and **g**) and DNs (**e**) were observed, and together with the cortical dysplasia, indicated the diagnosis of FCD type IIb. Frequent findings included: HyNs (open arrowhead in **a**), apical dendrites not oriented towards the pia surface (PS) (**b, f**); blurring of the grey-white matter transition (**a, g**); heterotopic neurons in the white matter (**h**), and intense gliosis, shown here in the vicinity of a cluster of large, unstained neurons (arrowheads) (**i**). Cases: 8, **a** and **e**; 10, **b, c, f, h**; 1, **d** and **g**; 11, **b, i**. H&E staining: **a, d, e, g**; SMI 311 antibody, **b, f, h**; silver impregnation, **c**; anti-GFAP antibody, **i**. Scale bars: 40 μm: **d, e**; 100 μm: **a, b, c, f, h, i**; 200 μm: **g**

arrow in 3 N), which are markers of neural progenitor cells and undifferentiated glia, respectively, and neuronal class III β-tubulin (Fig. 3c), which is an early neuronal marker. BCs also expressed GFAP (Fig. 3d), a mature glial marker, and neurofilament proteins (arrow in Fig. 3e), microtubule-associated protein type 2 (MAP-2) (Fig. 3f), beta-tubulin (Fig. 3g), and NeuN (arrow in Fig. 3i), which are adult neuronal markers. BCs were also stained by the SMI 311 antibody (Fig. 3h), a pan-neuronal marker. DNs expressed β-tubulin (Fig. 3j), NeuN (L), and overexpressed pan-neuronal filaments (3 K), which displaced nucleus towards cell periphery. DNs also expressed nestin

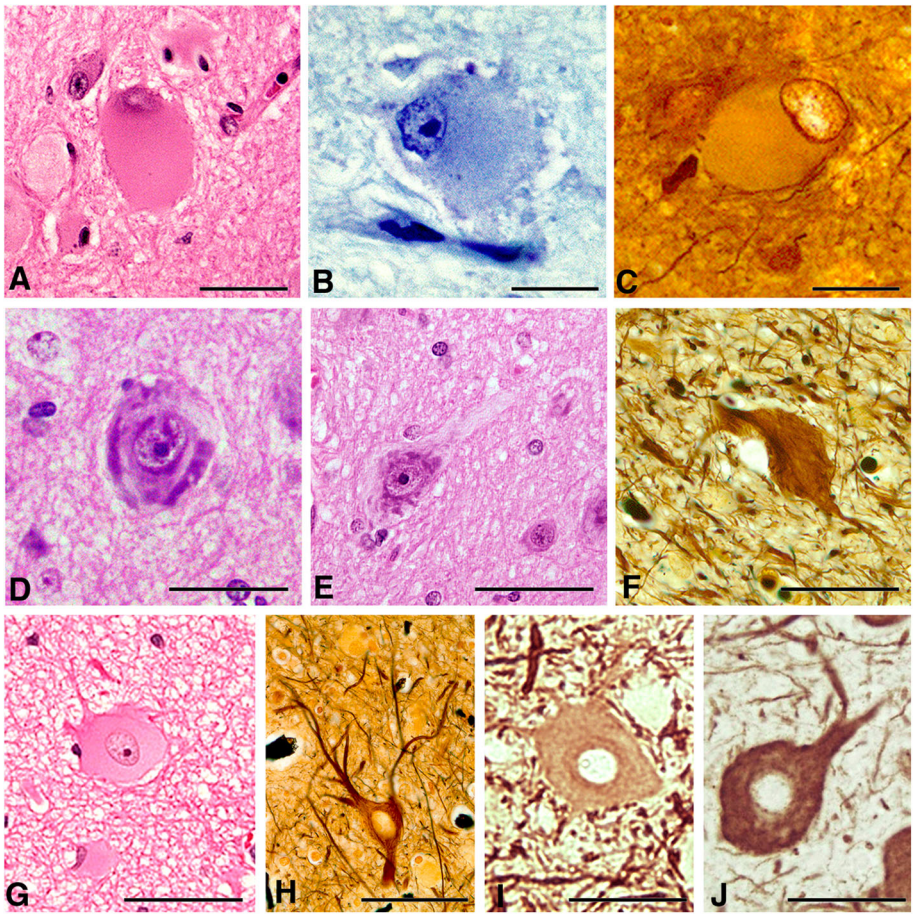

Fig. 2 Identification of balloon cells, dysmorphic and hypertrophic neurons. BCs presented large perikaria and eccentric nuclei upon H&E (**a**), cresyl violet (**b**) and silver staining by the Bielschowsky's method (**c**). Nucleolus was prominent (**b**), and the cytoplasm was homogeneous (**a, b, c**), glassy and eosinophilic by H&E staining, and without a detectable Nissl substance (**a**). DNs exhibited large cell bodies and nuclei (**d, e, f**), and Nissl substance was characteristically clumped or clumped and displaced towards the cell membrane (**d, e**). DNs neurons presented an eccentric nucleus, due to filament accumulation (**f**, silver impregnation). HyNs were as large as DNs, but their large nucleus was central (**g, h, i, j**), and Nissl substance was either not observed (**g**) or appeared as usual. HyNs were decorated by anti-neurofilament proteins (**i**) and SMI 311 antibodies (**j**), which are markers of mature neurons. Cases: 9, **a** and **b**; 1, **c**; 11, **d, f**; 16, **e**; 10, **g, i, j**; 8, **h**. H&E staining: **a, d, e, g**; Cresyl violet staining: **b**; silver impregnation: **c, f, h**; anti-neurofilament antibody: **i**; SMI 311 antibody, **j**. Bars, 30 μm: **a, b, c**; 40 μm: **d, e, f, g, i, j**; 100 μm: **h**

(arrowhead in Fig. 3m), vimentin (arrowhead in Fig. 3n), and class III β-tubulin (3O), which are markers of undifferentiated cells. Panel I (arrowhead) shows a HyN stained by the anti-NeuN antibody.

Discussion

Focal cortical dysplasias are malformations of cortical development that result from disturbances of developmental processes, which lead to cerebral cortex formation, and present with cortical dyslamination and abnormal cell morphology. These malformations are frequently associated with refractory epilepsies, and their diagnosis is essential to define the prognosis. The current work systematically documents the neuropathology of FCD type IIb, with a particular focus on the pathology and immunophenotype of DNs, HyNs, and BCs.

Identification of dysmorphic neurons, hypertrophic neurons, and balloon cells

BCs occur only in FCD type IIb. DNs occur in both FCD types IIa and IIb, but not in types I and III, whereas HyNs occur in FCD types, I, II and III (Palmini et al. 2004; Blümcke et al. 2011; Najm et al. 2018). DNs and HyNs are as large as or larger than layer V pyramidal neurons, and BCs are even larger cells. BCs and DNs present a large nucleus displaced to the cell periphery, whereas HyNs present a central nucleus. BCs often present several nuclei. Nissl substance stained by H&E or cresyl violet appears clumped, or clumped and displaced towards cell periphery in DNs, normal in HyNs, and absent in BCs. DNs are often bizarre structured neurons (Blümcke et al. 2009), whereas HyNs preserve a pyramidal morphology, with apical dendrites, and BCs

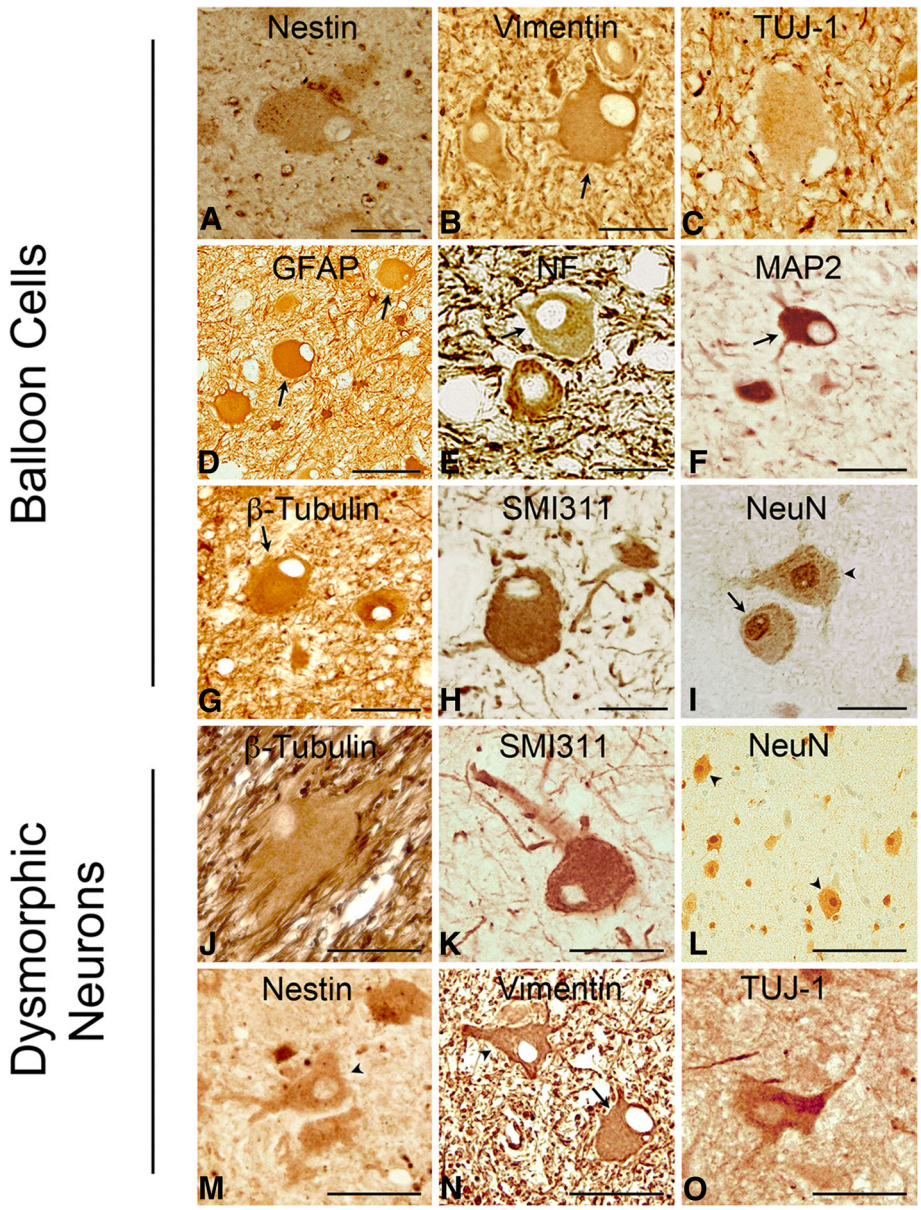

Fig. 3 Balloon cells, dysmorphic and hypertrophic neurons presented characteristics of undifferentiated and mature cells. BCs were decorated by antibodies against nestin (**a**), vimentin (**b**; arrow in **n**) and TUJ-1 (**c**), which are usually expressed by undifferentiated neural cells. BCs expressed GFAP at a variable intensity or not at all (**d**). BCs were also marked by antibodies against neurofilament proteins (arrow in **e**), MAP2 (**f**), β-tubulin (**g**), pan-neuronal filaments (**h**), and NeuN (arrow, **i**). DNs were stained by anti- β-tubulin (**j**), SMI 311 (**k**), and anti-NeuN (arrowheads in **l**). DNs were also marked by anti-nestin (**m**), anti-vimentin (**n**), and anti- neuronal class III β-tubulin (**o**). HyNs were stained with the anti-NeuN antibody (arrowhead in **i**). Cases: 10, **a, c, d, e, g, h, i, j, k, l, o**; 9, **b**, **m, n**; 5, **f**. Bars, 30 μm for all panels, except for panel (**d**), 50 μm

present a ballooned morphology. Silver impregnation of DNs by the Bielschowsky method shows a dense network of cytoplasmic fibrils, and BCs exhibit a characteristic golden-brown cytoplasm. The dense accumulation of phosphorylated or non-phosphorylated neurofilament proteins in the cytoplasm of DNs displaces the nucleus to the cell periphery, whereas neurofilament accumulation also occurs in HyNs, but without nucleus displacement, as shown here by SMI 311 and anti-neurofilament antibodies staining. Therefore, DNs and HyNs can be identified by the overexpression of neurofilament proteins, which occur in the DNs, but not in HyNs. The ILAE Commission (Blümcke et al. 2011) proposed the use of SMI 32 antibody to identify architecture disorganization in sections of cortex specimens but permitted the use of similar antibodies. The present report shows that the SMI 311 antibody can be used to stain the dysplastic cortex, normal and abnormal neurons in FCD IIb.

Immunophenotype of balloon cells, dysmorphic and hypertrophic neurons

Both DNs and BCs express nestin and vimentin, which are markers of neural progenitor cells and undifferentiated neural cells, respectively. However, only BCs express GFAP, a marker of mature glia. DNs and BCs also express class III β-tubulin, a marker of undifferentiated neurons, but they even express markers of mature neurons, which include NeuN, a nuclear marker, and β-tubulin and pan-neuronal filaments. A subpopulation of DNs presented a GABAergic profile (Cepeda et al. 2007; Cepeda et al. 2014). Thus, BCs expressed both markers of glia and neurons, and both BCs and DNs expressed markers of undifferentiated and differentiated neural cells, in agreement with previous reports (Orlova et al. 2010; Crino et al. 1996; Thom et al. 2005). However, there are different subpopulations of BCs and DNs, identified by panels of expressed epitopes (Ying et al. 2005; Lamparello et al. 2007). Some populations of these aberrant cells could be traced back to pluripotent stem cells, for example, by the expression of CD133 (Ying et al. 2005). Populations of BCs and DNs could be related to radial glia lineage (Lamparello et al. 2007), and could have resulted from abnormal proliferation, survival, migration and/or specification, in agreement with the hypothesis that BCs and DNs derived from radial glia (Lamparello et al. 2007; Englund et al. 2005; Cepeda et al. 2006), which can give rise to astrocytes and neurons during development (Rakic 1988; Noctor et al. 2001).

Molecular pathogenesis of FCD IIb

FCD type II, TSC and HME share several pathologies, which include DNs, HyNs, BCs and cortical dyslamination (Aronica and Mühlebner 2017; Najm et al. 2018; Aronica et al. 2012). However, in a large series of surgery patients with HME, BCs were identified in less than 50% of surgical specimens (Aronica and Mühlebner 2017). FCD IIb pathology is virtually indistinguishable from TSC tubers, and BCs are morphologically similar to the giant cells of TSC. TSC exhibits mutations of TSC1 (hamartin) and TSC2 (tuberin) genes leading to gene loss of function and activation of mammalian target of rapamycin complex (mTORC) (Ostendorf and Wong 2015). DNs (in FCD type IIa and IIb) and BCs (in FCD type IIb) also express aberrant mTORC activation (Lim et al. 2015), in addition to alteration of other protein pathways, such as Notch and Wnt, which are involved in neurogenesis, neuroglia cell fate, neuron migration and neural tube development (Cotter et al. 1999; Crino 2005). mTORC1 and mTORC2 are constitutively activated in BCs and DNs. mTORC1 promotes protein synthesis and controls cell size. mTORC2 regulates the actin cytoskeleton, determines cell morphology and controls dendritic growth. These two cascades could account, to some extent, for the abnormal cell phenotype of BCs and DNs (Ostendorf and Wong 2015). However, BCs and DNs also differ genetically from each other by differences in the mTOR activation cascade, which are reflected in the diverse expression of some of its components (Lim et al. 2015). The mTOR cascade is abnormally and constitutively activated in mTORpathies, which include TSC, FCD types IIa and IIb and HME. Genetic analysis can contribute to elucidate pathogenesis, differential diagnosis, and treatment of these mTORpathies (Ostendorf and Wong 2015; Crino 2011; Marsan and Baulac 2018).

Differential neuropathology diagnosis of FCD type II, TSC and HME

Brain pathology of FCD type II, TSC and HME present in common BCs, DNs and HyNs, and cortical dyslamination. The neuropathology differential diagnosis is based on: *first*, macroscopic examination of the HME brain often reveals an abnormal gyral pattern (pachygyria, polygyria, or polymicrogyria), as well as increased thickness of the cortex of the enlarged hemisphere, features that are not observed in FCD type II and TSC; *second*, TSC brain pathology is characterized by the presence of cortical tubers, subependymal giant-cell astrocytomas (SEGAs) and calcifications, which are useful to distinguish TSC from FCD type IIb and HME; *third*, both HME and TSC, but not FCD types IIa and IIb, can be associated with skin lesions; *fourth*, TSC, but not FCD type IIb and HME, is associated with a spectrum of hamartomas involving almost every organ in the body (Aronica and Mühlebner 2017). On the other hand, pathologies that present ballooned cells in the cerebral cortex but are not similar to FCD IIb, HME or TSC include pellagra and some neurodegenerative diseases (Ellison et al. 2004).

The present report presents limitations, which include the number of cases and number of antibodies to study immunophenotype.

Conclusion

Focal cortical dysplasias are malformations of cortical developments that result from disturbances of processes that lead to cerebral cortex formation. Focal cortical dysplasias present disorganization of the cerebral cortex architecture and abnormal cell morphology, are frequently associated with refractory epilepsy, and their post-surgical prognosis depends on their type. The diagnosis of the type of focal cortical dysplasia in a surgical specimen relies on the identification of the abnormal cells present within a dysplastic cortex, and the type of cortical disorganization. The present report contributes to the identification of balloon cells, dysmorphic and hypertrophic neurons, and their immunophenotype, in the context of focal cortical dysplasia type IIb.

Abbreviations
BC: Balloon cell; DN: Dysmorphic neuron; FCD: Focal cortical dysplasia; HME: Hemimegalencephaly; HyN: Hypertrophic neuron; SEGA: Subependymal giant cell astrocytoma; TSC: Tuberous sclerosis complex; TSC1: Tuberous sclerosis complex gene 1; TSC2: Tuberous sclerosis complex gene 2

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Availability of data and materials
The data generated and analysed in the current study are not publicly available due to ethical reasons, e.g., patient anonymity, but can be available from the corresponding author on reasonable request. Antibodies and chemicals used in this study are commercially available.

Authors' contributions
GKS, CSC, TCDD, TRV, VMA, and CABZ carried out experiments and reviewed data; VCT, HRM, JAA, CGC, JCDC, ALP, EP, ACS analyzed patient data and reviewed the manuscript; LN performed neuropathology diagnosis and reviewed the manuscript, RS and RG reviewed and contributed to the manuscript; and ARM coordinated the research, reviewed data, and wrote the manuscript with contributions from authors. All authors read and approved the final manuscript.

Authors' information
Not applicable.

Ethics approval and consent to participate
The Ethics Committees of our Institutions, Tissue Bank at Hospital das Clínicas, Ribeirão Preto Medical School, University of São Paulo, process 9370/2003, and Ethics Committee, Federal University of Triângulo Mineiro, process No. 1229, evaluated and approved the use of biopsies and the publication of the study results, on the basis of the informed, written consent given by the patients. Cases for this study were from patients with drug-resistant epilepsy who underwent surgical resection of the epileptogenic region for treatment.

Consent for publication
Patients consented for publication (process 9370/2003, Tissue Bank at Hospital da Clinicas, Ribeirão Preto Medical School, University of São Paulo, and process 1229, Ethics Committee, Federal University of Triângulo Mineiro and their data are maintained anonymous.

Competing interests
The authors declare that they have no competing interests.

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