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PDGFRβ$^+$ CELLS IN HUMAN AND EXPERIMENTAL NEURO-VASCULAR DYSPLASIA AND SEIZURES

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Abstract—*Introduction:* Neuro-vascular rearrangement occurs in brain disorders, including epilepsy. Platelet-derived growth factor receptor beta (PDGFRβ) is used as a marker of perivascular pericytes. Whether PDGFRβ$^+$ cell reorganization occurs in regions of neuro-vascular dysplasia associated with seizures is unknown. *Methods*: We used brain specimens derived from epileptic subjects affected by intractable seizures associated with focal cortical dysplasia (FCD) or temporal lobe epilepsy with hippocampal sclerosis (TLE-HS). Tissues from cryptogenic epilepsy, non-sclerotic hippocampi or peritumoral were used for comparison. An *in vivo* rat model of neuro-vascular dysplasia was obtained by pre-natal exposure to methyl-axozy methanoic acid (MAM). Status epilepticus (SE) was induced in adult MAM rats by intraperitoneal pilocarpine. MAM tissues were also used to establish organotypic hippocampal cultures (OHC) to further assess pericytes positioning at the dysplastic microvasculature. PDGFRβ and its colocalization with RECA-1 or CD34 were used to segregate perivascular pericytes. PDGFRβ and NG2 or IBA1 colocalization were performed. Rat cortices and hippocampi were used for PDGFRβ western blot analysis. *Results:* Human FCD displayed the highest perivascular PDGFRβ immunoreactivity, indicating pericytes, and presence of ramified PDGFRβ$^+$ cells in the parenchyma and proximal to microvessels. Tissues deriving from human cryptogenic epilepsy displayed a similar pattern of immunoreactivity, although to a lesser extent compared to FCD. In TLE-HS, CD34 vascular proliferation was paralleled by increased perivascular PDGFRβ$^+$ pericytes, as compared to non-HS. Parenchymal PDGFRβ immunoreactivity co-localized with NG2 but was distinct from IBA1$^+$ microglia. In MAM rats, we found pericyte-vascular changes in regions characterized by neuronal heterotopias. PDGFRβ immunoreactivity was differentially distributed in the heterotopic and adjacent normal CA1 region. The use of MAM OHC revealed microvascular-pericyte dysplasia at the capillary tree lining the dentate gyrus (DG) molecular layer as compared to control OHC. Severe SE induced PDGFRβ$^+$ immunoreactivity mostly in the CA1 region of MAM rats. *Conclusion:* Our descriptive study points to microvascular-pericyte changes in the epileptic pathology. The possible link between PDGFRβ$^+$ cells, neuro-vascular dysplasia and remodeling during seizures is discussed.

Key words: pericytes, PDGFRβ, seizures, neuro-vascular malformations.

INTRODUCTION

A number of CNS disorders are associated with developmental or progressive neuro-vascular damage. In particular, various extents of neuronal disorganization and disrupted microvascular architecture are reported in drug-resistant forms of epilepsy. In addition to neuronal and endothelial cells, the pathophysiological role of a number of perivascular and parenchymal cells in CNS disorders is gaining momentum (Armulik et al., 2005; Bergles et al., 2010; Friedman, 2011; Prinz et al., 2011; Marchi and Lerner-Natoli, 2013). Among those, pericytes are mural cells outlining the microcapillary wall, controlling vascular integrity (Armulik et al., 2010; Bell et al., 2010; Winkler et al., 2011). The platelet-derived growth factor receptor beta (PDGFRβ), a tyrosine kinase receptor regulating pericyte-vascular interplay, is used as a marker of pericytes (Winkler et al., 2010, 2011; Olson and Soriano, 2011). PDGFRβ immunoreactivity is also reported in the brain parenchyma (Fernandez-Klett et al., 2013; Shepherd et al., 2013). PDGFRβ$^+$ cells may have phenotypic plasticity and, depending on the developmental stage, were proposed as progenitor cells, perivascular fibroblast or mesenchymal stem cells with differentiation capacity and immune functions (Olson and Soriano, 2011; Hewitt et al., 2012; Fernandez-Klett

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Abbreviations: DG, dentate gyrus; FCD, focal cortical dysplasia; FCDIIb, Type IIb focal cortical dysplasia; MAM, methyl-axozy methanoic acid; OHC, organotypic hippocampal culture; PDGFRβ, platelet-derived growth factor receptor beta; ROI, region of interests; SE, status epilepticus; TLE-HS, temporal lobe epilepsy with hippocampal sclerosis.

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et al., 2013; Matsumoto et al., 2014; Birbrair et al., 2015). Interestingly, PDGFRβ signaling is involved in the initiation of fibrogenesis in the brain and in non-CNS organs (Borkham-Kamphorst et al., 2004; Makihara et al., 2015).

Recent evidence has indicated PDGFRβ$^+$ cells' reactivity in the epileptic brain (Shepherd et al., 2013; Milesi et al., 2014). Pericyte redistribution around the microvasculature was observed after severe status epilepticus (SE) in mice (Milesi et al., 2014), while disarray of the pericyte-basal lamina interface occurs in human epilepsy (Liwnicz et al., 1990). Whether modification of the pericyte-vascular assembly or changes in PDGFRβ cell distribution corresponds to neuronal heterotopias associated, or not, to seizures is unclear. The latter is significant considering the contribution of pericytes to microvascular integrity (Dore-Duffy, 2008; Daneman et al., 2010; Winkler et al., 2011). Increased cerebrovascular permeability and dysregulated angiogenesis provoke or sustain seizures in experimental and human epilepsy (Seiffert et al., 2004; Marchi et al., 2007; Rigau et al., 2007; van Vliet et al., 2014).

In the present study we refer to perivascular PDGFRβ$^+$ cells as pericytes (Armulik et al., 2005; Dore-Duffy, 2008; Winkler et al., 2011), while not specifying the exact nature of parenchymal PDGFRβ immunoreactivity (Birbrair et al., 2013, 2014a,b, 2015; Fernandez-Klett et al., 2013; Matsumoto et al., 2014). We have evaluated the morphology and positioning of PDGFRβ$^+$ cells in human cortical specimens and hippocampi (TLE) resected from drug-resistant epileptic subjects. Neocortex presenting focal cortical dysplasia (FCD) was compared to cryptogenic epilepsy, where no dysplasia is reported or to peritumoral specimens. Pericyte-vascular dysplasia and PDGFRβ reactivity to severe SE were evaluated using a rat model of neuronal heterotopia obtained by pre-natal exposure to methyl-axozy methanoic acid (MAM; Colacitti et al., 1998, 1999). Finally, we used a novel organotypic hippocampal culture (OHC) established from MAM hippocampi to further characterize pericyte distribution in regions of vascular dysplasia.

EXPERIMENTAL PROCEDURES

Patients' selection and immunohistochemistry

Nineteen samples were selected retrospectively from the epilepsy surgery and pathology database at the Neurological Institute Carlo Besta, Milan (see Table 1). Samples derived from patients affected by drug-resistant focal epilepsy who previously underwent surgery after a comprehensive electro clinical and MRI evaluations (Tassi et al., 2010). Eight hippocampi (four with sclerosis, HS, and four with not sclerotic), three non-malformed cerebral cortices (cryptogenic) and eight malformed cerebral cortices presenting Type IIb focal cortical dysplasia (FCDIIb) were used (see Table 1). Peritumoral cortices (distant from the lesions and not presenting morphological alterations or vascular proliferation) previously obtained from four patients without history of epilepsy were used as controls (not listed in Table 1). All of resections were performed for therapeutic reasons. Surgically resected brain specimens (5–7 mm thickness) were immersed in 4% paraformaldehyde for 24 h at 4 °C and then cut (50-μm serial sections) using a Vibratome (VT1000S; Leica, Heidelberg, Germany). Alternate sections adjacent to those processed for immunohistochemistry were stained with Thionin (0.1%) to obtain cytoarchitectonic maps. Briefly, neuropathology (Table 1) was confirmed using: monoclonal anti-NeuN (1:3000, Chemicon, Temecula, CA, USA), pan-neuronal specific for non-phosphorylated neurofilaments (SMI311R, 1:1000, Covance, San Diego, CA, USA); monoclonal anti-GFAP (1:15,000, Chemicon) and monoclonal antibody against intermediate filament protein vimentin (VIM, 1:3000, Dako, Carpinteria, CA, USA) to diagnose balloon cells in Type IIb FCD. Sections were incubated with a rabbit monoclonal anti-PDGFRbβ (Abcam, 1:100, Cambridge, UK), or a mouse monoclonal anti-CD34 (1:100, Dako, Carpinteria, CA, USA) to visualize blood microvessels, and a mouse monoclonal anti chondroitin sulfate NG2 (1000, Pharmingen, New Jersey, USA). Standard immunoperoxidase procedures were performed as previously described (Garbelli et al., 1999). Fluorescent double immunostaining was performed using a mixture of primary antibodies (PDGFRβ/CD34; overnight at 4C) and subsequently incubated with the corresponding secondary antibodies indocarbocyanine Cy3-conjugated goat anti rabbit (1:600) and Cy2-conjugated goat anti mouse (1:200) (Jackson Immunoresearch Laboratories, West Grove, PA, USA) for 2 h at room temperature. PDGFRβ/NG2 colocalization was performed using the primary anti-PDGFRβ and secondary Cy3-conjugated goat

Table 1. Patients' data. FCD = focal cortical dysplasia; F = frontal; ID = identification number of the patient; na = not available; P = parietal; T = temporal. Peritumoral samples are not included

	ID	Age at seizure onset (years)	Duration of epilepsy (years)	Monthly seizure frequency	Site of surgery
Hippocampi					
No HS	1	na	na	na	T
	2	17	18	3	T
	3	21	12	7	T
	4	18	6	10	T
HS	5	8	27	6	T
	6	11	26	30	T
	7	9	19	4	T
	8	14	25	4	T
Cortices					
Cryptogenic	9	18	6	10	T
	10	8	20	13	T
	11	17	18	3	T
FCD	12	2	2	300	P
	13	8	3	20	T
	14	7	3	na	F
	15	3	36	15	T
	16	8	39	45	F
	17	6	40	76	P
	18	1	33	16	F
	19	2	43	20	F