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FCD Type II and mTOR pathway: Evidence for different mechanisms involved in the pathogenesis of dysmorphic neurons

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ABSTRACT

Type II focal cortical dysplasia (FCD II) is a malformation of cortical development, frequently associated with intractable epilepsy, characterised by cortical dyslamination, dysmorphic neurons (DNs) and balloon cells (BCs). We investigated the expression of pS6 (downstream target) and pPDK1-pAkt (upstream targets) as evidence for mTOR pathway activation and their co-expression with Interleukin-1β in FCD II surgical specimens and compared the findings with control non-epileptic tissue, non-malformed epileptic tissue or acquired epilepsy-Rasmussen's Encephalitis (RE) occasionally presenting pS6 and Interleukin-1β positive abnormal neurons. Downstream mTOR activation was demonstrated in almost all abnormal cells in both FCD II and RE. Conversely, upstream activation in FCD II was observed in the majority of BCs, in a proportion of DNs, not presenting Interleukin-1β expression, but not at all in RE scattered abnormal neurons. Based on these findings we suggest that the presence of BCs and DNs in FCD II could be due to a first upstream mTOR pathway PI3K-Akt-mediate event occurring very early during cortical development in the large proportion of abnormal cells; followed by the appearance of additional pS6 positive DNs promoted by the presence of a later inflammatory processes.

1. Introduction

The mTOR pathway is a cellular signaling cascade involved in the pathogenesis of numerous malformations of cortical development (MCDs). In the case of Tuberous Sclerosis Complex (TSC), a developmental disorder characterised by benign hamartomas in multiple organs and tubers in the brain, mTOR hyperactivation has a defined origin: the loss of function mutations in *TSC1/TSC2* genes directly involved in mTOR regulation (Tavazoie et al., 2005; Crino et al., 2006). Over the last ten years, a number of studies have demonstrated the abnormal expression of mTOR downstream targets in other developmental disorders whose clinicopathological features such as epilepsy, intractable seizures, developmental delay, and altered cortical architecture are similar to TSC (Baybis et al., 2004; Miyata et al., 2004; Ljungberg et al., 2006; Boer et al., 2007; Crino, 2011).

The consequences of mTOR pathway activation mainly consist of severe cortical alterations with abnormal cell morphology, frequently associated with intractable epilepsy. These neuropathological alterations may be due to the aberrant expression of various downstream mTOR proteins that are not normally activated during cortical development (Tsai et al., 2014) because mTOR acts as a central regulator of many functions during development and maturation, including cell growth and proliferation, energy metabolism, inflammation and synaptic plasticity (Laplante and Sabatini, 2012; Meng et al., 2013; Yasin et al., 2013).

Type II focal cortical dysplasia (FCD II) is a localised cortical malformation characterised by profound alterations in cortical architecture and the presence of cytological abnormalities. The most recent ILAE classification (Blumcke et al., 2011), similarly with the previous one (Palmini et al., 2004), recognises two FCD II subtypes: Type IIa based on the presence of dysmorphic neurons

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Table 1
Clinical and neuropathological data.

ID	Age at seizure onset (years)	Age at surgery (years)	Monthly seizure frequency	Site of surgery	Neuropathological diagnosis	Outcome (Engel class)
1	0	1	300	TO	FCD IIa	Ia
2	10	29	30	T	FCD IIa	IV
3	2	4	100	F	FCD IIa	Ia
4	0	13	60	O	FCD IIa	Ia
5	1	9	600	FP	FCD IIa	Ia
6	4	29	2	T	FCD IIa	Ia
7	0	31	60	TO	FCD IIa	Ic
8	0	25	30	TPO	FCD IIb	Ia
9	3	39	30	T	FCD IIb	Ia
10	2	45	20	F	FCD IIb	Id
11	2	3	10	F	FCD IIb	IIb
12	4	22	40	T	FCD IIb	Ia
13	10	14	100	F	FCD IIb	II
14	0	13	1	F	FCD IIb	Ia
15	1	12	300	T	FCD IIb	Ia
16	0	4	300	F	FCD IIb	Ia
17	1	34	16	F	FCD IIb	Ia
18	6	46	75	P	FCD IIb	Ib
19	4	31	90	F	FCD IIb	na
	2.6 (3)	**21.3 (14.4)**	**113.9 (154.5)**			
20	5	13	150	F	RE	Ia
21	2	4	300	TF	RE	Ia
22	5	6	300	TF	RE	Ia
	4 (1.7)	**7.8 (4.7)**	**250 (86.6)**			
23	26	30	12	T	Cryptogenic	Ia
24	25	36	12	T	Cryptogenic	Ia
25	11	27	15	T	Cryptogenic	III
26	3	8	90	T	Cryptogenic	Ia
27	1	21	60	T	Cryptogenic	II
28	4	13	30	T	Cryptogenic	Ia
29	3	36	6	T	Cryptogenic	Ib
	10.4 (10.7)	**24.4 (10.9)**	**31.1 (31.4)**			

Legend: FCD = Focal Cortical Dysplasia; F = Frontal; ID = identification number of the patient; na = not available; O = Occipital; P = Parietal; RE = Rasmussen's Encephalitis; T = Temporal.
In bold are expressed the mean values (standard deviation).

(DNs) alone, and Type IIb in which DNs are encountered together with balloon cells (BCs). Abnormal activation of proteins related to downstream (Baybis et al., 2004; Miyata et al., 2004; Ljungberg et al., 2006; Schick et al., 2007) and upstream mTOR pathway, particularly the PI3k-Akt (Ljungberg et al., 2006; Schick et al., 2006; Schick et al., 2007; Lin et al., 2015), has been reported in both DNs and BCs and mutations of genes belonging to this pathway have been found. In fact, studies documenting pathogenic germline and somatic mutations in the mTOR gene or in other genes including GATOR1-encoding complex (DEPDC5, NPRL2, NPRL3), PI3KCA and AKT3 have been identified in some patients (Baulac et al., 2015; Baulac, 2016; Lim et al., 2015; Nakashima et al., 2015; Jansen et al., 2015).

In FCD II, DNs, defined as enlarged neurons with abnormal aggregate of neurofilaments and Nissl substance in cytoplasm (Blumcke et al., 2011), are considered hallmarks of FCD II and TSC but similar cells are also occasionally observed in some acquired epilepsy-associated pathologies, including hippocampal sclerosis (HS) and Rasmussen's encephalitis (RE) (Prayson, 2012; Rogerio et al., 2014). The expression of phospho-S6 (pS6), a downstream target of the mTOR pathway, has been detected in these abnormal cells, alternatively termed either hypertrophic or dysmorphic neurons (Liu et al., 2014), but the mechanisms inducing this activation have not yet been defined. Nevertheless, it is well known that mTOR pathway hyperactivation leads to a constellation of changes such as adult neurogenesis in the hippocampus, somatic and dendritic hypertrophy, enhanced synaptic transmission and plasticity believed to promote epileptogenesis and hyperexcitability (Lasarge and Danzer, 2014). mTOR has also been shown to be involved in the regulation of both the innate and adaptive immune responses (Soliman, 2013). Studies investigating the disruption of the mTOR signaling could help to define the potential pathogenetic role of this pathway in a number of epileptic diseases and possibly identify new therapeutic targets.

On the basis of these premises, the aim of this study was to investigate the patterns of mTOR pathway expression in a cohort of patients with Type IIa and Type IIb FCD, particularly: 1) the relationship between upstream and downstream compartments; 2) the spatial distribution of the activated targets in the BCs and DNs in the core of the lesion, and 3) the contribution of inflammatory processes to mTOR pathway hyperactivation. Non-epileptic peritumoral tissue and tissues from patients with cryptogenic epilepsy or acquired epilepsy-associated pathology were investigated in the same manner for comparative purpose. Correlations between mTOR pathway expression and genetic analysis are beyond the scope of the present paper.

2. Material and methods

2.1. Patients and specimen selection

The analysed human cortical samples were obtained from a total of 32 patients:29 patients with drug-resistant epilepsy and 3 non-epileptic patients with brain tumours all surgically treated at the Department of Neurosurgery of C. Besta Neurological Institute and the C. Munari Epilepsy Surgery Centre of Niguarda Hospital (both in Milan, Italy). The examined surgical specimens were removed for strictly therapeutic reasons after informed consent. Tissue was