DR MARIA THOM (Orcid ID : 0000-0001-7712-2629)

PROFESSOR THOMAS S JACQUES (Orcid ID : 0000-0002-7833-2158)

Article type : Editorial

Novel therapeutic targets in epilepsy: oxidative stress and iron metabolism

Yao-Feng Li[1], Maria Thom[2], Thomas S. Jacques[3,4]

Pathology Department, Tri-Service General Hospital & National Defence Medical Centre, Taipei, Taiwan, ROC[1]

Department of Neuropathology, UCL Institute of Neurology, London, UK[2]

Developmental Biology and Cancer Research & Teaching Department, UCL Great Ormond Street Institute of Child Health, London, UK[3]

Departments of Histopathology, Great Ormond Street Hospital NHS Foundation Trust, London, UK[4]

E-mail: liyaofeng1109@gmail.com

Author for correspondence:

Professor Thomas S Jacques

UCL Great Ormond Street Institute for Child Health, 30 Guilford Street, London WC1N 1EH, UK

Tel: +44 (0)20 7829 8663

Fax: +44(0)20 7831 4366

Email: t.jacques@ucl.ac.uk

Key words: epilepsy, focal cortical dysplasia type IIb (FCDIIb) and Tuberous Sclerosis complex (TS), Oxidative stress (OS), Nrf-2, iron metabolism.

Malformations of Cortical Development (MCD) are one of the most frequent causes of multidrug-resistant focal epilepsy, of which focal cortical dysplasia type IIb (FCDIIb) and Tuberous Sclerosis (TS) are two of the commonest lesions encountered (1, 2). TS cortical lesions (cortical tubers) and

FCD IIb have been long recognised to share histological similarities; a recent leap in our understanding, confirming their relationship, has been the identification of low level somatic mutations in mTOR (mechanistic target of rapamycin) pathway genes in FCD types (3). The term 'MTORopathies' has been coined for this group (4) as a disease continuum, and mutations have now been confirmed in up to 46% of FCD lesions (5) but with mTOR activation evident in virtually all TS/FCDIIb cases by demonstration of phosphorylation of downstream protein targets such as pS6. mTOR is a master regulator of many critical cellular metabolic processes, including attenuation of inflammatory responses and cell survival. Experimental studies support its influence over the cytopathology and migratory abnormalities observed in these developmental lesions (6). However, how mTOR initiates or interacts with the processes leading to epileptogenesis is a more elusive, complex and critical question with likely 'cross talk' between multiple cellular pathways. In the recent study from the Van Vliet and Aronica laboratories, Zimmer *et al.* explore cellular alterations in the mTORopaties using multiple experimental paradigms, focusing on the interactions between seizures, mTOR activation, pro-inflammatory mechanisms, miR155 expression and the effects on cellular oxidative stress (OS) and iron metabolism(7).

OS is a self-propagating phenomenon involving reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive lipid species (RLS), inducing lipid peroxidation and cellular damage with 4-hydroxy-2-nonenal (4-HNE) production (8). When OS occurs, endogenous antioxidant mechanism (such as Nrf-2 signalling) prevent cell damage and death (8). OS is also closely related to neuroinflammation (9) and a growing body of evidence also implicates neuroinflammatory mechanisms in FCDIIb and TS (10-14). In a previous recent study from this group, they found co-existence of neuroinflammatory markers (TLR4, COX2, and NF-κB) and OS markers (xCT, iNOS) in

FCDIIb and TS (15) and they have also recognised microRNAs as being key players in the regulation of inflammatory responses in TS, in addition to mTOR (16).

The current study explores the relationships between OS, miR155 and mTOR and confirms that there is lipid peroxidation (4-HNE) and DNA double-strand damage (γH2A.X) in pS6-positive lesional cells, providing evidence of OS-related damage. Notably, they observed evidence of OS in hippocampal astrocytes and increased miRNA155 in the hippocampus and cortex, even before seizure onset in a TS mouse model (*Tsc1*GFAP-/-). Using astrocyte cultures, they demonstrated that overexpression of miR155 increases susceptibility to OS, and induced OS-reactive and iron regulatory genes, likely influencing free-iron availability.

This circle of experiments then led them back to explore the question of iron metabolism in surgical TS and FCDIIb cases, and they identified increased ferritin in lesional cells, albeit with some heterogeneity, but again with a correlation with mTOR activation. Similar findings were also confirmed in their TS mouse model, again at a phase before seizure onset, indicating both OS and iron dysregulation as early events. This is also in keeping with previous work showing inflammation occurs before seizures in the TSC brain (13) and mouse models (17). They also found 4-HNE reactivity and ferritin in neuronal precursors in fetal TS tissues and upregulation of both antioxidant and iron regulatory genes shown in an independent TS cohort. They showed that prolonged activation of Nrf-2 from OS in mTORopathies leads to accumulating of intracellular iron and propose that the disturbance in iron metabolism further exacerbates OS. Moreover, as Nrf-2 signalling plays an essential role in mediating iron metabolism (18), although the short term activation of Nrf-2 signalling could help balance the OS and prevent cellular damage by inducing antioxidant mechanism paradoxically the long term effects could cause iron

overloaded and exacerbate lipid peroxidation and ferroptosis (8).

Of key importance in this unravelling of complex cellular interactions in the mTORopathies is the exposure of potential new druggable targets in the early stages of epileptogenesis. Most current anti-epileptic drugs control seizures only. Therefore, mTORopathies are largely drug-refractory and surgery is currently the best treatment option for FCDIIb with post-operative rates of seizure freedom of around 32-66% (19). Future personalised treatments, precisely targeting aberrant cells and pathways, are the ultimate goals. Broad spectrum mTOR inhibitors show promise (20) but have limitations and side effects with long-term use. Zimmer *et al.* propose that agents inhibiting iron adaptation, such as Nrf-2 inhibitors or ferroptotic agents, may represent alternative future strategies in the intervention of aberrant pre-epileptogenic cellular processes in these disorders and therefore seizure prevention.

Conflicts of interest

The authors declare no conflicts of interest relevant to this publication.

Author contribution

All authors contributed to writing this editorial.

Funders

As an editorial, there is no direct funding for this work. However, TSJ acknowledges funding from the Brain Tumour Charity, Children with Cancer UK, Great Ormond Street Hospital (GOSH) Children's Charity, Olivia Hodson Cancer Fund, Cancer Research UK and the National Institute of Health Research (NIHR). Our work is supported by the INSTINCT network funded by The Brain

Tumour Charity, GOSH Children's Charity and Children with Cancer UK, and the EVEREST centre funded by The Brain Tumour Charity. All research at GOSH NHS Foundation Trust and UCL Great Ormond Street Institute of Child Health is made possible by the NIHR GOSH Biomedical Research Centre. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

References

1. Blumcke I, Spreafico R, Haaker G, Coras R, Kobow K, Bien CG, et al. Histopathological Findings in Brain Tissue Obtained during Epilepsy Surgery. The New England journal of medicine. 2017;377(17):1648-56.

2. Orlova KA, Crino PB. The tuberous sclerosis complex. Annals of the New York Academy of Sciences. 2010;1184:87-105.

3. Benova B, Jacques TS. Genotype-phenotype correlations in focal malformations of cortical development: a pathway to integrated pathological diagnosis in epilepsy surgery. Brain pathology. 2019;29(4):473-84.

4. Jansen LA, Mirzaa GM, Ishak GE, O'Roak BJ, Hiatt JB, Roden WH, et al. PI3K/AKT pathway mutations cause a spectrum of brain malformations from megalencephaly to focal cortical dysplasia. Brain : a journal of neurology. 2015;138(Pt 6):1613-28.

5. Marsan E, Baulac S. Review: Mechanistic target of rapamycin (mTOR) pathway, focal cortical dysplasia and epilepsy. Neuropathology and applied neurobiology. 2018;44(1):6-17.

6. Kim JK, Lee JH. Mechanistic Target of Rapamycin Pathway in Epileptic Disorders. J Korean Neurosurg Soc. 2019;62(3):272-87.

7. Zimmer TS, Ciriminna G, Arena A, Anink JJ, Korotkov A, Jansen FE, et al. Chronic activation of anti-oxidant pathways and iron accumulation in epileptogenic malformations. Neuropathol Appl Neurobiol. 2020 Jan 14;29:5926.

8. Dodson M, Castro-Portuguez R, Zhang DD. NRF2 plays a critical role in mitigating lipid peroxidation and ferroptosis. Redox biology. 2019;23:101107.

9. Geronzi U, Lotti F, Grosso S. Oxidative stress in epilepsy. Expert review of neurotherapeutics. 2018;18(5):427-34.

10. Boer K, Crino PB, Gorter JA, Nellist M, Jansen FE, Spliet WGM, et al. Gene expression analysis of tuberous sclerosis complex cortical tubers reveals increased expression of adhesion and inflammatory factors. Brain Pathol. 2010;20(4):704-19.

11. Iyer A, Zurolo E, Spliet WG, van Rijen PC, Baayen JC, Gorter JA, et al. Evaluation of the innate and adaptive immunity in type I and type II focal cortical dysplasias. Epilepsia. 2010;51(9):1763-73.

12. Mills JD, Iyer AM, Van Scheppingen J, Bongaarts A, Anink JJ, Janssen B, et al. Coding and small non-coding transcriptional landscape of tuberous sclerosis complex cortical tubers: Implications for pathophysiology and treatment. Sci Rep. 2017;7(1).

13. Prabowo AS, Anink JJ, Lammens M, Nellist M, Van Den Ouweland AMW, Adle-Biassette H, et al. Fetal brain lesions in tuberous sclerosis complex: TORC1 activation and inflammation. Brain Pathol. 2013;23(1):45-59.

14. Zhang Z, Liu Q, Liu M, Wang H, Dong Y, Ji T, et al. Upregulation of HMGB1-TLR4 inflammatory pathway in focal cortical dysplasia type II. Journal of neuroinflammation. 2018;15(1):27.

15. Arena A, Zimmer TS, van Scheppingen J, Korotkov A, Anink JJ, Muhlebner A, et al. Oxidative stress and inflammation in a spectrum of epileptogenic cortical malformations: molecular insights into their interdependence. Brain pathology (Zurich, Switzerland). 2019;29(3):351-65.

16. van Scheppingen J, Iyer AM, Prabowo AS, Muhlebner A, Anink JJ, Scholl T, et al. Expression of microRNAs miR21, miR146a, and miR155 in tuberous sclerosis complex cortical tubers and their regulation in human astrocytes and SEGA-derived cell cultures. Glia. 2016;64(6):1066-82.

17. Zhang B, Zou J, Rensing NR, Yang M, Wong M. Inflammatory mechanisms contribute to the neurological manifestations of tuberous sclerosis complex. Neurobiol Dis. 2015;80:70-9.

18. Kerins MJ, Ooi A. The Roles of NRF2 in Modulating Cellular Iron Homeostasis. Antioxidants & redox signaling. 2018;29(17):1756-73.

19. Veersema TJ, Swampillai B, Ferrier CH, van Eijsden P, Gosselaar PH, van Rijen PC, et al. Long-term seizure outcome after epilepsy surgery in patients with mild malformation of cortical development and focal cortical dysplasia. Epilepsia open. 2019;4(1):170-5.

20. Curatolo P, Franz DN, Lawson JA, Yapici Z, Ikeda H, Polster T, et al. Adjunctive everolimus for children and adolescents with treatment-refractory seizures associated with tuberous sclerosis complex: post-hoc analysis of the phase 3 EXIST-3 trial. Lancet Child Adolesc Health. 2018;2(7):495-504.