

Balloon cells associated with granule cell dispersion in the dentate gyrus in hippocampal sclerosis

M. Thom · L. Martinian · L. O. Caboclo ·
A. W. McEvoy · S. M. Sisodiya

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Abstract Granule cell dispersion (GCD) is a common finding in hippocampal sclerosis in patients with intractable focal epilepsy. It is considered to be an acquired, post-developmental rather than a pre-existing abnormality, involving dispersion of either mature or newborn neurones, but the precise factors regulating it and its relationship to seizures are unknown. We present two cases of GCD with associated CD34-immunopositive balloon cells, a cell phenotype associated with focal cortical dysplasia type IIB, considered to be a developmental cortical lesion promoting epilepsy. This observation opens up the debate regarding the origin of balloon cells and CD34 expression and their temporal relationship to seizures.

Keywords Granule cell dispersion · Hippocampal sclerosis · Balloon cells · CD34

Introduction

Focal cortical dysplasia (FCD) and hippocampal sclerosis (HS) are distinct pathologies associated with refractory focal epilepsy. Balloon cells (BCs) are considered a pathognomonic feature of FCD type IIB [24]. Abnormal but variable expression of intermediate filaments, including glial fibrillary

acidic protein (GFAP), as well as developmentally regulated proteins [6, 11, 14, 30] have culminated in the popular view that BCs are a manifestation of compromised cortical development, involving deranged cell migratory and differentiation pathways [5]. Recently, we and other groups have demonstrated membranous expression of stem cell marker CD34 on BCs in around 60% of FCD cases [14, 26].

Conversely, HS is more generally regarded as an acquired abnormality, although the presence of an underlying maldevelopmental template is often debated. Granule cell dispersion (GCD) of the dentate gyrus is present in around 40% of cases with HS [28], and contemporary theories as to its aetiology include a migration of mature granule cells triggered by local gliosis and reelin deficiency or alternatively seizure-enhanced granule cell genesis [12, 27]. We now report the finding of CD34 expression in BC-like cells in association with GCD in the context of isolated HS raising further conjecture regarding cyto-architectural anomalies in focal epilepsies.

Case history

Patient 1

This patient was a 43-year-old right-handed woman with epilepsy since the age of 12. She was born after an uneventful pregnancy and had no perinatal problems. She achieved normal milestones without any antecedent central nervous system (CNS) infection, head injury or febrile seizures. Her father had a past history of generalised seizures, well controlled with antiepileptic drugs. She had simple and complex partial seizures with rare generalised seizures. MRI showed reduction in right hippocampal volume with mild signal change with concordant video-electroencephalogram

M. Thom (✉) · L. Martinian · L. O. Caboclo · A. W. McEvoy ·
S. M. Sisodiya
Department of Clinical and Experimental Epilepsy,
Institute of Neurology and National Hospital for Neurology
and Neurosurgery, Queen Square, London WC1N 3BG, UK
e-mail: M.Thom@ion.ucl.ac.uk

M. Thom
Department of Neuropathology, Institute of Neurology,
Queen Square, London WC1N 3BG, UK

(EEG) findings. The patient underwent a right anterior temporal lobectomy. In the first year following surgery, she had a few auras, but no seizures with loss of consciousness.

Patient 2

This patient was a 35-year-old right-handed woman with epilepsy since the age of 7. There was no history of perinatal problem of any nature, and no familial history of epilepsy or febrile seizures. She had simple and complex partial seizures (up to 4/day), refractory to treatment but no generalised seizures. MRI revealed clear-cut severe diffuse left HS, along with extensive damage particularly in cortical regions in the left frontal lobe, involving most of this lobe. On neuropsychological evaluation, learning and memory for a word list were clearly impaired, consistent with dominant temporal involvement. In addition, word finding difficulties and speech and reading errors were apparent and consistent with more widespread albeit dominant hemisphere involvement, though a frontal syndrome was not in evidence. Non-verbal memory was intact. Depth electrodes studies confirmed a mesial temporal lobe origin for the seizures. The patient underwent left anterior temporal lobectomy. Two years after surgery, she remains free of all seizures, still taking anti-epileptic drugs (AEDs).

Pathology

The resected hippocampi were routinely fixed and processed. In addition to routine H&E and Nissl/luxol fast blue (LFB) stains, immunohistochemistry was carried out briefly as follows. Seven micrometer paraffin-embedded sections were cut, dewaxed, rehydrated through graded alcohols and taken to water. Endogenous peroxidase was blocked with 3% hydrogen peroxide in deionised water for 10 min. Sections were microwaved with antigen retrieval solution (Vector Burlingame, CA, USA). Sections were incubated with following polyclonal primary antibodies: Dynorphin dilution 1:100 (AbD Serotec), GFAP (1:1,500 Dako), GFAP-delta (1:5,000 Chemicon), NeuN (1:1,000 Chemicon), Nestin (1:8,000 Chemicon), Neurofilament (1:500 2F11 Dako), Neuropeptide Y (NPY) (1:4,000 Sigma), mcm2 (1:900 BD Biosciences) and vimentin (1:2,000 Dako) and counterstained with haematoxylin.

In case 1, the typical pattern of classical HS with marked neuronal loss from hilar, CA1 and CA3 subfields with relative sparing of CA2 [8] was identified (MTS type 1 [2]). At some levels, the hilar region appeared rarefied with loss of myelin. The granule cell layer showed focal marked dispersion of NeuN-positive neurones into the molecular layer (Fig. 1a). The dispersed cells appeared fusiform in shape with mild cytomegaly, variation in nuclear size and accentu-

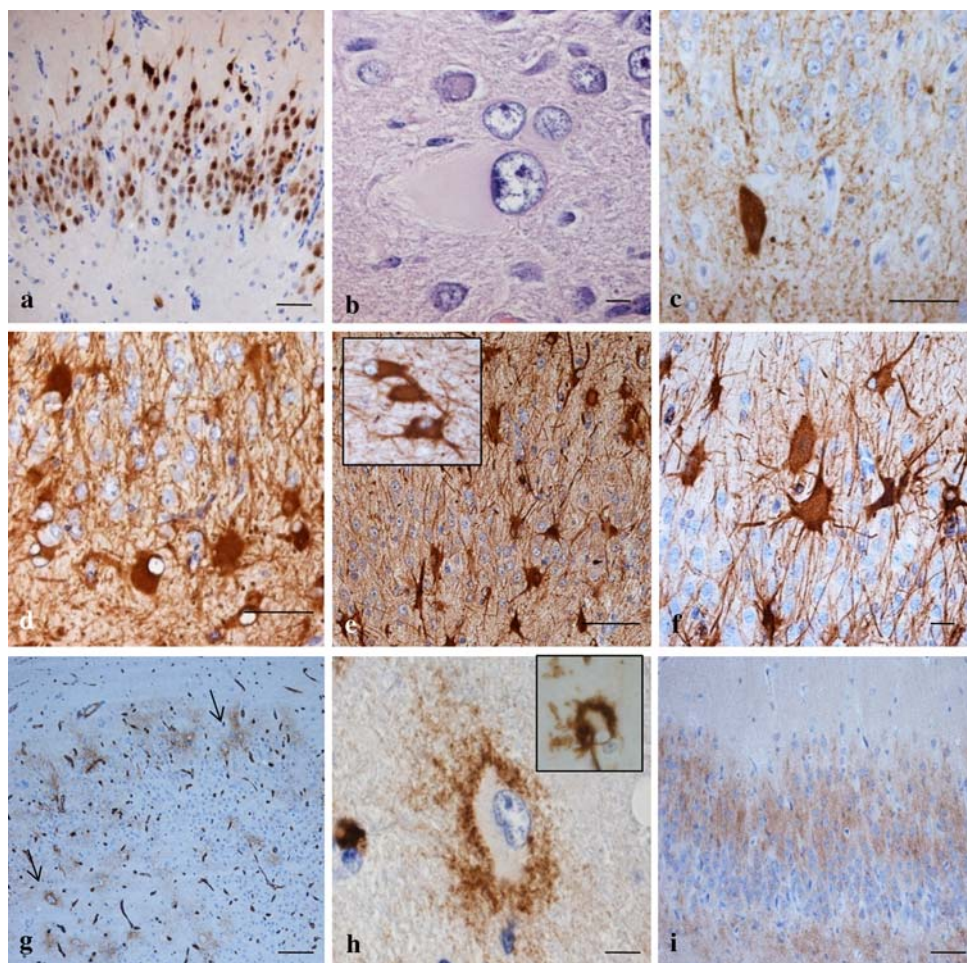
ated, coarse Nissl staining of cytoplasm. Interspersed between the granule cells, in the sub-granular zone and molecular layer were astrocytic cells with ballooned cell morphology with abundant glassy eosinophilic cytoplasm (Fig. 1b). These cells were mainly positive for GFAP (Fig. 1d), although some cells stained less intensely and many were positive with GFAP-delta (Fig. 1e) and vimentin (Fig. 1f). Many showed stunted processes whilst others displayed longer radial processes extending through the granule cell layer. A proportion of these cells were highlighted by CD34 immunostaining with a ruffled peripheral border of immunoreactivity (Fig. 1g, h), many with radiating processes reminiscent of patterns of CD34 positivity in BCs in FCD. Anti-nestin antibodies labelled occasional multipolar cells in the subgranular zone, but the BCs did not label with nestin or with mcm2 proliferation marker as we have previously shown in BCs in FCD IIB [26]. The displaced granule cells were negative for CD34 but positive for dynorphin with evidence of mossy fibre sprouting in the molecular layer (Fig. 1i). Conversely, the ballooned astrocytes did not label with NeuN, dynorphin or NPY. Examination of the temporal lobectomy specimen did not reveal evidence of FCD IIB.

In case 2, the findings were similar but the brunt of the hippocampal neuronal loss was focused on the end folium with marked rarefaction of the hilar region (MTS type 3 [2]). There was generalised severe dispersion of granule cells, which appeared hyperchromatic on Nissl stain and intermingled with glassy, ballooned astrocytes that were GFAP-positive. Neurofilament stains showed scattered enlarged neurones in the region of the dentate gyrus (Fig. 1c). Dynorphin was confined to the granule cells in the dentate gyrus with no mossy fibre sprouting; NPY patterns were as described in HS but did not label the BCs [8]. The BCs also showed focal CD34 membranous positivity (Fig. 1h). CD34 immunostaining was carried out in 20 further cases of HS with GCD and varying degrees of astrogliosis, but no similar cases were identified.

Discussion

CD34 is a stem cell marker expressed on haemopoietic progenitor cells as well as mature vascular endothelium, with roles in cell adhesion and cell signalling [16]. Blumcke demonstrated the expression of CD34 in low-grade tumours, particularly gangliogliomas and malformative pathologies arising in the temporal lobe in patients with resistant focal epilepsy [1]. In Blumcke's initial study, only 1 of 25 cases with HS was observed to show solitary staining of cells in CA1 with CD34 immunohistochemistry, and no labelling in the dentate gyrus was reported. Subsequently, CD34 expression was also demonstrated in BCs associated with FCD IIB, with cells demonstrating a

Fig. 1 **a** NeuN staining in case 1 confirming marked dispersion of the granule cells. **b** “Balloon cell” with glassy cytoplasm in the dentate gyrus on H&E. **c** Neurofilament immunohistochemistry showing enlarged neurone in the dentate gyrus. **d** GFAP immunohistochemistry confirming balloon-like glial cells in DG as also observed with **e** GFAP-delta antibodies (also shown in inset in other case) and **f** vimentin immunohistochemistry. **g** CD34 immunostaining highlighted a number of these ballooned cells in the region of the dentate gyrus (*arrows*), which showed a peripheral rim of cytoplasmic labelling in case 1 (**h**) (*inset*: morphologically similar cell shown in case 2). **i** Dynorphin immunohistochemistry confirming mossy fibre sprouting into the molecular layer. (Bars in **a, c, d, e, h, g, i** = 100 μ m; **b, f** = 10 μ m)



membranous staining pattern [14] interpreted to represent abnormal differentiation. In the present cases, we demonstrate CD34-positive cells with the morphology of BCs in the context of HS with GCD.

GCD is a common finding, seen in around 40% of HS cases [2]. The displaced neurones in the molecular layer may appear enlarged and fusiform, as in the present cases [10, 17, 20, 21] reminiscent of migrating neurones [23], and more rarely their axis is malorientated. A radial gliosis typically accompanies GCD [12]. Dysmorphic hilar neurones and interneurones have been described in HS cases previously [4, 7, 22, 25, 29] but not BCs. In the context of FCD IIB, BCs are accompanied by dysmorphic and hypertrophic neurones within a dyslaminal cortex. Morphologically similar cells are also observed in cortical lesions associated with tuberous sclerosis and hemimegalencephaly (HMG) but not with other tumours or malformations. One explanation proposed for BCs in FCD IIB is that they represent radial glia, which have failed to degenerate following cortical maturation [5]. Recently, GFAP-delta expression has been shown in BCs of FCD, an isoform associated with progenitor cell glia [18], and similar expression was observed in the BCs in the present cases.

The identification of CD34-positive balloon-like cells in GCD in HS raises several possibilities. This might represent a primary “Taylor-like” dysplasia localised to the dentate gyrus. The development of the dentate gyrus [19] is generally distinct from corticogenesis. The cause of FCD is unknown; however, some signalling pathways implicated, such as Wnt pathway [6], are also involved in the morphogenesis of dentate gyrus. Alternatively, CD34 may be transiently expressed in the process of GCD, perhaps related to enhanced neurogenesis and glial progenitor cell proliferation. It is difficult to prove that GCD in human TLE is a result of migration of new neurones. In most cases, the end-stage of the disease is being studied in surgical tissue. Enhanced cell proliferation has been shown in association with GCD [27], although low levels of doublecortin expression [12] support the view that dispersed neurones have a mature, differentiated phenotype. As it is increasingly recognised that CD34 has roles in cell adhesion, glial expression of this epitope may also be relevant to the process of GCD [16]. Indeed, one theory proposes that the neuronal migration is facilitated by the radial glial scaffold extending through the dentate gyrus [12, 15]. However, as CD34 expression in both ours and previous series of HS [1]

appears to be infrequent, a significant role for CD34 in the process of GCD would seem unlikely. Indeed, as CD34 positivity shows variable expression in a spectrum of malformative and low-grade tumour pathologies associated with epilepsy [1, 3, 9], both in cells of glial and probable neuronal lineage [13], expression promoted by seizures could also be a plausible explanation. These present cases raise interesting questions for future studies in the regulation of CD34 expression in lesional epilepsy pathology.

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