The enlarging spectrum of focal cortical dysplasias

This scientific commentary refers to 'PI3K/AKT pathway mutations cause a spectrum of brain malformations from megalencephaly to focal cortical dysplasia', by Jansen et al. (doi: 10.1093/brain/awv045).

Focal malformations of cortical development, including focal cortical dysplasia (FCD), are among the most common causes of intractable epilepsy in children. In this issue of Brain, Jansen and colleagues provide evidence that hemimegalencephaly (HMEG) and FCD type IIa result from mosaic mutations in components of the PI3K/Akt/mTOR (mammalian target of rapamycin) pathway, i.e. PI3KCA, AKT3, or PTEN, in 4/33 patients using targeted screening of a panel of PI3K, AKT, and PTEN genes (Jansen et al., 2015). These individuals suffered from intractable seizures, and exhibited radiographic and histopathologically defined evidence of either HMEG or FCD. An additional single case of dysplastic megalencephaly (DMEG) was linked to PI3KCA. In the remaining 29 patients with HMEG, or FCD type I, type IIa, or type IIb, no mutations were identified. The phosphorylation profile of components of the PI3K/Akt/mTOR signalling pathway showed that phosphorylation of Akt at threonine (T)308 and serine (S)473 was enriched in all HMEG and FCD cases, and in conjunction with measured Akt kinase activity, could distinguish mutation-positive dysplasia cortex, mutation-negative dysplasia cortex, and non-dysplasia epilepsy cortex. In conclusion, Jansen et al. suggest that FCD, HMEG and DMEG comprise a spectrum of developmental malformations linked to the PI3K/Akt/mTOR pathway.

Focal malformations of the cerebral cortex have been reported since the 1800s, with the first description of HMEG by Sims in 1835 and tuberous sclerosis complex by Bourneville in 1880. In 1957, Crome published a short pathological report describing abnormally ‘large neurons’ in the brains of three patients with intractable epilepsy within areas of ‘ulegyria’, which set the conceptual stage for the index report of focal dysplasia of the cerebral cortex 14 years later by Taylor et al. (1971). Since neuroimaging had not yet been invented, these early studies relied on histopathological descriptions of large neurons, astrocytosis, and giant or ‘balloon-like’ cells. These cellular features would be critical to understanding the pathogenesis of FCD and would provide clues to some of the cell signalling abnormalities now linked to FCD four decades later. Pathological classification schemes have divided FCDs into types I–III based on distinct histopathological features. In particular, FCD type II (a and b) exhibit the most dramatic alterations in cytoarchitecture with cellular enlargement and dysmorphism. There is no widely accepted classification scheme for HMEG, but there is clearly a spectrum of radiographic and histopathological abnormalities in this disorder.

The molecular and developmental pathogenesis of HMEG and FCDs has come under close scrutiny in the past decade. The initial insights came from studies demonstrating enhanced signalling of the mTOR pathway in tuberous sclerosis complex (TSC) and FCDIIb (Baybis et al., 2004; Miyata et al., 2004; Ljungberg et al., 2006). These studies showed enhanced phospho-activation of the mTOR effectors p70S6kinase and ribosomal S6 protein in resected FCD and TSC tuber specimens. The critical link was that loss of function mutations in either TSC1 or TSC2, the genes responsible for TSC and known upstream inhibitors of mTOR, caused constitutive activation of mTOR and thus, these early studies linked FCDIIb with the mTOR cascade. Subsequent studies confirmed aberrant mTOR signalling in HMEG in a pattern that was similar to TSC and FCDIIb (Ljungberg et al., 2006; Aronica et al., 2007). Early speculations based on gene expression profiles suggested that while TSC, FCDIIb, and HMEG shared some histopathological similarities (noted by Taylor et al., 1971), they did not result from identical molecular mechanisms, a notion that has been borne out by molecular analysis. The distribution of cells exhibiting enhanced mTOR activation suggested a cell autonomous profile e.g. enriched in cytomegalic neurons and balloon cells, and in conjunction with a sporadic occurrence, suggested that HMEG and FCD resulted from somatic gene mutations, potentially in PI3K/Akt/mTOR pathway regulatory genes. This has since been confirmed, with the robust mTOR signalling abnormalities in these disorders linked to somatic mutations in genes including PI3KCA, PI3KR and AKT, as well as MTOR in HMEG (Lee et al., 2012; Poduri et al., 2012; D’Gama et al., 2015). Somatic PTEN and AKT mutations have been identified in small FCD cohorts, while recent reports have described new FCD syndromes linked to mutations in DEPDC5 (Baulac et al., 2015), a component of the mTOR regulatory GATOR-1 complex. Jansen and colleagues suggest that so-called ‘hot-spot’ mutations in PI3KCA and AKT are pivotal in HMEG pathogenesis. The evidence for PI3KCA as a common causative gene in FCD is less robust since only a single FCDIIa case in their cohort exhibited a PI3KCA mutation (a missense change). No mutations were identified in FCDI or FCDIIb specimens. However, given that only a targeted gene panel screen was used, many other genes within the PI3K/Akt/mTOR pathway could be responsible for FCDIIb, including MTOR itself.

The second portion of the paper examines phospho-activation of
components of the PI3K/Akt/mTOR signalling pathway. At least five previous studies have demonstrated clearly that both HMEG and FCD exhibit activation of this pathway (Baybis et al., 2004; Ljungberg et al., 2006; Aronica et al., 2007; Schick et al., 2007). However, Jansen et al. provide new data regarding functional activation of the PI3K/Akt/mTOR pathway as a consequence of PI3KCA, AKT3, and PTEN mutations using immunohistochemical, western, and in vitro kinase assays. In the PI3KCA, AKT3 and PTEN mutation cases, there is enhanced phosphorylation of Akt at T308 (a PI3K-driven signalling event mediated by PDK1) and S473 (driven by mTOR; also shown by Schick et al., 2007) consistent with pathway activation. In vitro Akt kinase assays demonstrated functional activation of Akt signalling as a consequence of PI3KCA, AKT, and PTEN mutations. Phosphorylation of ribosomal protein S6 at Ser 235/236 was found in virtually all cases analysed, both with and without identified mutations, suggesting that mTOR activation is a constant feature of FCD and HMEG.

The concept that FCD and HMEG represent a spectrum has been posited previously (Crino, 2007) and is now borne out by solid cell and molecular biological evidence. The evolving view is that a number of distinct germline or somatic mutations (or both), in genes encoding components of the PI3K/Akt/mTOR pathway, will account for many if not all forms of HMEG and FCD. However, the notion of a spectrum, in truth, reflects phenotypic variability. In this report, PI3KCA mutations were associated with HMEG and FCD type IIa and in effect, the findings of Jansen et al. suggest that HMEG may be actually a hemispheric FCDIIa. How does PI3KCA cause the range of malformations spanning FCD to HMEG? Although the identified mutations all lead to some degree to activation of PI3K/Akt/mTOR pathway signalling, the associations between lesion size or severity and pathway activation are not linear. For example, PI3KCA mutations were associated with only mildly enlarged neuronal size, whereas AKT3 mutations were associated with dramatically enlarged neuronal size. Akt signalling in the small FCDIIa (12-251-PI3KCA) case in which allelic variants were detected at 4.7% is actually 20% higher than the much larger HMEG cases 11-443-AKT3, 12-241-PI3KCA, and 12-123-PTEN in which allelic variants were detected at 10–18%, 31% and 50%, respectively. From a clinical translational perspective these data would suggest that there is more avid PI3K/Akt/mTOR activation in the smaller FCD lesion, perhaps requiring more aggressive pharmacological kinase inhibition for a clinical trial to be successful. We must now confront the factors that account for the wide disparities in lesion size, gyral patterning abnormalities, and heterogeneous cellular constituents characteristic of FCD and HMEG. For example, in addition to differential activation of PI3K/Akt/mTOR signalling shown by in vitro kinase assays, the timing of the somatic mutation e.g. early versus
later in foetal brain development, the progenitor cell type sustaining a somatic mutation, or the number of progeny derived from the affected progenitor cell, could dictate lesion size. We currently have little understanding of when somatic mutations occur specifically and in what subpopulations of progenitor cells. Perhaps some progenitor cells with greater mitotic capacity give rise to HMEG while others lead to smaller FCDs. To date, only single somatic mutations have been detected in FCD and HMEG, thus arguing for a clonal expansion from a single cell sustaining a mutation to form the lesion. The detected allelic frequencies in Jansen’s report range from 4.7% in the FCDIIa to 50% in HMEG; clearly there is a greater mutational burden in HMEG compared to FCD. Non-cell autonomous effects of the mutation, for example, altered release of neurotransmitters or trophic substances from affected cells onto surrounding progenitor cells may also contribute to variable lesion features.

From a mechanistic perspective, the overarching assumption at this point is that PI3KCA, AKT3, and PTEN mutations enhance pathway signalling. In theory, activating mutations in PI3KCA and AKT or inactivating mutations in PTEN, should signal downstream to mTOR through Akt mediated-phospho-inhibition of TSC2, culminating in a common pathway causing altered brain development, dysfunctional network circuitry in a localized or hemispheric distribution, and epileptogenesis. Now, the interesting study by Jansen et al. in the context of several other recent reports, begs a compelling question: while we know the mechanism for activation of the PI3K/Akt/mTOR pathway, how do these changes lead to the pathogenesis of these disorders and more importantly, to intractable epilepsy? PI3K, Akt, and mTOR are not, by themselves ‘end game’ proteins. Rather, as kinases, they serve to signal and pass information to functional effectors downstream. There are numerous potential Akt substrates besides TSC2 e.g. PRAS40, GSK3, FOXO, that govern a multitude of cellular processes including cell growth, cell survival, metabolism, and angiogenesis, that could be pivotal to FCD and HMEG pathogenesis. Similarly, mTOR governs the translation of numerous cellular proteins and serves to phospho-activate or inhibit a large number of substrates. Future studies must critically examine downstream signalling effects in each of these disorders with a wary eye on genotype-phenotype correlations. Indeed, the terms ‘FCD’ and ‘HMEG’ may be inadequate to capture the full complexity of each malformation type. There is an enormous amount of work now needed to investigate the profiles of neurotransmitter receptor and channel expression, inflammatory changes, and cell lineage in FCD and HMEG in the context of specific genotypes. Indeed, while the promise of clinical therapies predicated on manipulation of, for example mTOR signalling may be appealing, precision medicine approaches to epilepsy in these disorders may require still more targeted therapies.

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