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Juvenile Myoclonus Epilepsy: Dual role for EFHC1 in the Etiology?

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Abstract. Mutations in EFHC1 gene causes juvenile myoclonic epilepsy (JME) which is the most common of idiopathic generalized epilepsy, accounting for 7% of all epilepsies. Being a non-ion channel gene, function studies of the EFHC1 protein have revealed the three different physiological functions of this protein. Firstly, EFHC1 thought to modulate calcium channel and cause programmed cell death in the developing brain. Secondly, EFHC1 was shown to be a microtubule-associated protein and found to be involved in the regulation of cell division. Thirdly, mutant analysis of Defhc1 in Drosophila exhibited a number of neuronal defects, including abnormal synaptic development and morphogenesis. This commentary looks at the available in the literature on EFCH1 and the etiology of epilepsy; evolve a hypothesis as how defects in a non-ion channel gene could underlie epileptic symptoms, and why JME should be considered as a developmental disorder.

Keywords: Epilepsy, Channelopathy, Non-ion channel genes, Cell migration, Calcium sensor

1. Introduction

Epilepsy is complex neurological disorder characterized by seizures and affecting 50 million people worldwide [1, 2]. Seizures results from abnormal electrical activity in the neuron and neuronal electrical discharges are regulated by ion channels [3]. Thus defects in genes coding for ion channel proteins is one of the genetic cause of idiopathic forms of epilepsy [4].Thus epilepsy syndromes are often grouped under channelopathies [3-6]. One of the exceptions to the concept of "channelopathies" in epileptic syndromes is the mutations in the EFHC1 gene, a non –ion channel gene causing JME in significant number of families [7-11]. Juvenile myoclonic epilepsy is one of the most common epilepsy syndromes accounting for 7% of all cases of epilepsy [2, 12], and is classified as one of the idiopathic epilepsies - a group characterized by absence of detectable form for brain lesions [1, 2].

The JME gene EFHC1 codes for a protein Myocloni1/ EFHC1 which is ubiquitously expressed and an EF- hand containing calcium binding protein [8, 13]. EFHC1 gene makes two transcripts resulting from differential splicing of exon 4 [8]. The two protein isoforms coded by these two transcripts, where minor isoform lacks the EF-hand motif. JME associated mutations have been found in both types of transcripts [8, 11].

JME inheritance is autosomal dominant and so far all the EFHC1 mutations show heterozygosity in affected individuals [8]. Nevertheless whether JME phenotypes results from gain or loss of function of mutant EFHC1 protein is yet to be clearly explained. Few functional studies on the EFHC1 protein using *in vitro* and *in vivo* models have explained new physiological functions of this protein and led to two different models for epileptogenesis. This commentary highlights the recent finding of EFHC1 gene functions and its connection to the genesis of epilepsy.

2. JME Aetiology - Current Models

2.1. EFHC1, Calcium Signalling and Apoptosis

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The EFHC1 gene was found to be associated with JME on chromosome region 6p12–p11 in year 2004. EFHC1 encodes 640 amino acid proteins and consists of three DM10 domains, a motif with unknown function and an EF hand, a Ca2+ binding motif. EFHC1 was found to localize in the cell body and dendrites on the neuron [8, 14]. Overexpression of EFHC1 resulted in shorter and fewer branches of dendrite and the neuron undergo degeneration and leading to apoptosis [8]. Intriguingly, Overexpression of EFHC1 mutant protein in the neurons did not show such abnormal neuronal features and apoptosis is significantly lowered by EFHC1 mutations. Therefore, it is being suggested that EFHC1 mutations might have affected some of the functions of EHHC1 and one of such function could be positively regulating the apoptosis of the neurons [7, 8]. The EFHC1 protein EF-hand domain has been shown to bind to calcium [8, 13]. This binding of calcium induces a conformational change in the EF hand domain-containing proteins which, in most cases, result in the changes in the functions of the target proteins involved often in catalyzing an enzymatic reaction [15]. Therefore, it is important to test whether the EFHC1-induced apoptosis observed in the neuronal cultures is mediated through the calcium channels. It was found that overexpression of EFHC1 in the presence of calcium channel blockers increased the survival rate of neurons. Therefore confirming the hypothesis that EFHC1-induced neuronal apoptosis is mediated through calcium signalling [8]. Indeed EFHC1 was found to positively regulate activity of calcium channels, and the increased influx of calcium ions could cause the neuronal cell death [8]. Thus, it could be concluded that the JME linked mutations in the EFHC1 might affect the apoptotic process in the brain and that absence of neuronal apoptosis could underlie epileptic symptoms in JME patients [7, 8].

This conclusion leads to an intriguing question; why should neurons undergo apoptosis for a brain to be healthy? And an intriguing answer comes from our understanding of the developmental processes.

Programmed cell death – the alternate name for apoptosis, is an important event during embryogenesis and also development of its central nervous system. Nearly 70% of the developing neurons undergo apoptosis at various stages for the morphogenetic events [16]. Thus loss or mutants of EFHC1 might affect this process and brain may end up having additional and unwanted neurons in unwanted places which results in the hyper excitable circuits causing epileptic seizures [7].

The authors tested the other proteins involved in this process and found EFHC1interacts with transient receptor potential M2 channel (TRPM2) Ca2+-permeable cation channel. The EFHC1 and TRPM2 were co localized in neurons. EFHC1 positively regulates the activity of TRPM2 and the susceptibility to cell death and thus TRPM2 could also contribute to JME epileptic symptoms by partially suppressing the deleterious effects of JME mutations in EFHC1 on the cellular processes such as apoptosis [17].

2.2. EFHC1, Cell Divsison, Neuroblast Migration Andsynapse Dendrite Formation

Cell biological assays on the EFHC1 protein led to its new functions in cell division. Firstly, EFHC1 protein was found to be associated with mitotic cycle. EFHC1 protein was recruited to mitotic spindle, and the centrosome [18]. EFHC1 protein sub-cellular localization varied during the mitotic cycle, hence it was suggested that EFHC1 could be a microtubule associated protein, and could be involved in mitotic cell division [18]. The EFHC1 gene is expressed maximally in the brain during its embryonic development –a stage where cell division is at maximum, suggesting that EFHC1 could possibly be involved in cell cycle regulation in the developing brain [8, 18]. This leads to a second intriguing question; what could be the connection between cell cycle and JME? Some recent studies show that neuronal cytoskeletal machinery modulates the cell cycle of the neuronal progenitors, and that the proliferation of the progenitor cells in the developing cortex is essential for proper neurogenesis, neuronal migration and axonal wiring [19, 20]. Since these three events – neurogenesis, neuronal migration, and axonal wiring – are dynamic in nature, and are regulated by microtubule associated proteins, a causal role for EFHC1 in the proliferation of neuronal progenitors and migration was proposed and tested [21]. Firstly, it was shown that overexpression of truncated versions of the EFHC1 in a human kidney cell line resulted in the impaired mitotic spindle defects, and chromosomes misalignments during the metaphase, and apoptosis [21].Conversely, RNAi-mediated of knock down of EFHC1 in the same cell line led to an increase in the mitotic index, suggesting that EFHC1 negatively modulates M-phase progression [21]. Secondly, knockdown of EFHC1 in developing cortical region severely affected the neuronal migration. This defect could possibly be due to the fact that the

progenitor cells that were devoid of EFHC1 did not exit the cell-cycle and continued to proliferate mitotically [21].Mutant analysis of Defhc1 loss- and gain-of-function alleles in vivo in Drosophila revealed a number of neuronal defects, including abnormal synaptic development characterized by extensive satellite bouton formation, increased frequency of spontaneous neurotransmitter release, and aberrations in dendritic arbour morphogenesis. Taken together these results have demonstrated that EFHC1 is a regulator of cell division and neuronal migration during cortical development synaptic bouton and dendritic morphogenesis and defects in this process lead to JME , hence now being considered as a as a developmental disease [22].

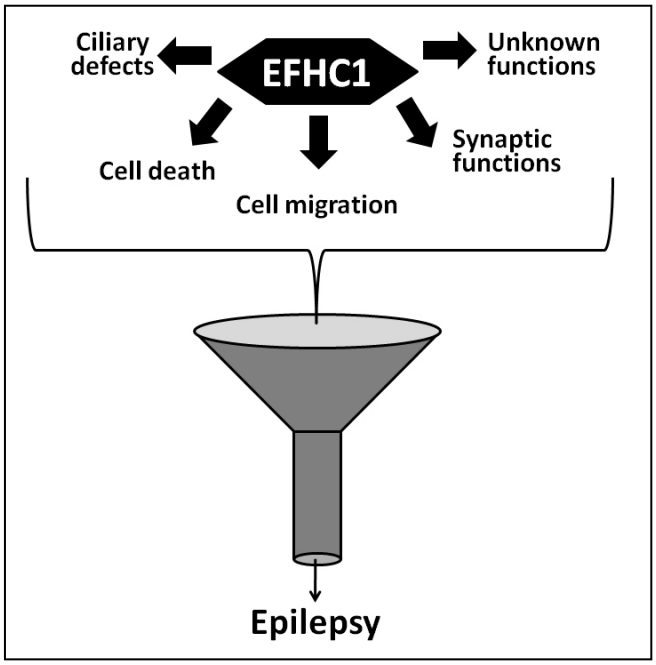

Fig. 1: Schematic diagram showing the proposed functions of EFHC1 and how defects in most of them result in the epileptic phenotype in JME.

2.3. JME - A Developmental Disorder?

The recent functional studies on genes associated with the diseases like JME have discovered novel links between common cellular processes and neurological disorders. The proposed functions of the EFHC1 proteins – that (i) it promotes apoptosis, (ii) it regulates the neuronal differentiation and their migration and that (iii) it regulates synaptic functions – may appear to be apparently unconnected. It is also equally likely that EFHC1 may perform some unknown function, defects in which result in the epileptic phenotype (see Fig. 1). Notwithstanding such discovery, none of the known roles of EFHC1 supports an age old concept that epilepsy results from primary defects in the ion channels, suggesting that defective channel functions are end effect, and may not be the primary cause for the epilepsy. It could be argued that, at least in JME, the structural changes in brain to be an underlying cause for the epileptogenesis, and that the JME could be a developmental disorder that manifest in the adolescence. The recent discoveries on EFHC1 question the very concept that the JME is one of the idiopathic epilepsies – that is epilepsy without structural lesions in the brain. Thus, symptomatic treatment may not be the best approach for treatment. The effective therapy should start much early, and should aim at restoring the functions of EFHC1 - at least partially - to overcome the debilitating the symptoms associated with JME possibly resulting from the abnormal brain development. With emerging knowledge in brain development and function, and further studies on the EFHC1 gene, the next few years should witness a significant improvement in our understanding on the cellular functions of EFHC1 protein, and how defects in this process may lead to some of the symptoms seen in JME. Thus further studies are required to understand the cellular targets of EFHC1 and the pharmacological targets, both of which are likely to be non-ion channels. Such efforts might help us in developing effective strategies for early interventions and therapeutics. Thus, paradigm shift in our approach to control epileptic seizures is required to bring-in smile to the affected.

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