CDKL5 UK study

This is an excellent review which has been updated from its previous version to reflect the up-to-date research that has been conducted all over the world into CDKL5 disorder. We are very thankful to Dr David Millar, from Cardiff University who is currently working on a project for CDKL5 UK looking at the Molecular Defects in the CDKL5 gene.

Introduction

CDKL5 is a serine-threonine kinase whose deficiency causes the early-onset variant of Rett syndrome. CDKL5 has been implicated in a number of different processes including alternative splicing, neuronal morphogenesis and dendritic arborisation, and energy metabolism. This article reviews the current knowledge regarding the molecular defects that give rise to this severe and debilitating disease.

Clinical features

As well as classical Rett syndrome (RTT; OMIM 312750), a number of different variant forms of RTT have been described including the early-onset seizure variant first described by Hanefeld in 1985 (Hanefeld 1985). Subsequently, Kalscheuer et al. (2003) found that mutations in the the cyclin dependent kinase-like 5 (CDKL5) gene (Montini et al. 1998) caused severe X-linked infantile spasms and mental retardation. Subsequently, mutations in the same gene were found in patients with clinical symptoms mimicking Rett syndrome (RTT). To date, more than 100 cases of CDKL5 deficiency have been reported in the literature. As this is an X-linked disorder, the majority of cases seen are in girls but approximately 10% are in boys. The clinical phenotype seems to be more severe in boys (Fehr et al. 2015). Several consistent clinical features have emerged including: (i) a normal prenatal history; (ii) irritability and drowsiness in the perinatal period; (iii) early-onset epilepsy with onset before 5 months of age; (iv) Rett-like features consisting of deceleration of head growth, stereotypies, poor to absent voluntary hand use, and sleep disturbances, and (v) severe mental retardation with poor eye contact and virtually no language (reviewed by Bahi- Buisson and Bienvenu 2012). The early onset seizures seen in this form of RTT (early-onset seizure variant of Rett syndrome or ESV RTT) have been shown to evolve through three stages, consisting of (1) early epilepsy, followed by (2) infantile spasms and finally (3) multifocal and refractory myoclonic epilepsy (Bahi- Buisson et al. 2008). The clinical phenotype seen in patients with CDKL5 mutations has recently been reviewed by a number of authors (Bahi- Buisson and Bienvenu 2012; Fehr et al. 2012; Kilstrup-Nielsen et al. 2012). Fehr et al. (2012) have shown that a significant number of patients with CDKL5 gene mutations have a clinical phenotype which does not meet the latest criteria for ESV RTT (Neul et al. 2010). These authors suggest that these patients with CDKL5 disorder should be considered separate from RTT, rather than another variant.

Another important observation in patients with CDKL5 disorder with confirmed CDKL5 gene mutations is that they have increased levels of 4-hydroxynonenal protein adducts (a marker of oxidative stress) in their peripheral blood lymphocytes (Pecorelli et al. 2011). This finding was also seen in patients with MECP2 gene mutations but not those with FOXG1 mutations (Pecorelli et al. 2011), suggesting

that oxidative stress, possibly as a result of mitochondrial dysfunction, may play a role in the pathogenesis of CDKL5 disorder. Pecorelli et al. (2015) have subsequently shown perturbed cholesterol homeostasis and decreased levels of the high density lipoprotein receptor SRB1. In addition they also found that one of the main protective mechanisms against oxidative stress, activation of the transcription factor NFE2L2, is impaired. CDKL5 deficient fibroblasts have both reduced basal protein levels of NFE2L2 and a reduced NFE2L2 response to oxidative stress (Pecorelli et al. 2015).

Leoncini et al. (2015) demonstrated that there was cytokine dysregulation in both classical RTT and CDKL5 disorder. They showed that cytokine dysregulation was proportional to clinical severity, inflammatory status and redox imbalance. The authors suggest that both classical RTT and CDKL5 disorder are associated with subclinical immune dysregulation caused by a defective inflammatory regulatory signalling system. The observed cytokine changes were partially corrected by omega-3 PUFAs (Leoncini et al. 2015).

Thyroid function has also been investigated in patients with CDKL5 disorder. Stagi et al. (2015) showed that patients had a higher level of free T4 than controls. However, at the present time, the clinical significance of these results is not clear.

Apart from the severe neurological features associated with CDKL5 disorder there are a number of other physiological deficits that have been found in patients. Although the significance of these defects is unclear at the present time it is important to recognise that CDKL5 disorder impacts on other physiological systems that may impact on patient health.

Gene Structure and Expression

Following the identification of the human CDKL5 gene (Montini et al. 1998), a number of different isoforms and splicing variants have been identified. The CDKL5 gene is made up of 24 exons of which the first three (exons 1, 1a, 1b) are untranslated; the remaining 21 exons contain the coding sequence. Two splice variants with different 5' untranslated regions have been found: isoform I contains exon 1 and is transcribed in a wide range of tissues, whereas isoform II, which includes exons 1a and 1b, is expressed only in the testis and fetal brain (Kalscheuer et al. 2003; Williamson et al. 2012). The original CDKL5 transcript generates a protein of 1030 amino acids (CDKL5115; 115kDa) which is expressed in the testis. Two other recently identified transcripts are likely to be relevant for CDKL5 brain function (Fichou et al. 2011; Rademacher et al. 2011; Williamson et al. 2012). Firstly, an alternatively spliced isoform giving rise to a novel in-frame exon (exon 16b) of 123 bases between exons 16 and 17 (Fichou et al. 2011; Rademacher et al. 2011). Whether this variant causes any change in function of the CDKL5 protein remains unclear but Fichou et al. (2011) demonstrated that the amount of the exon 16b-containing (CDKL5-16b) transcript varied depending upon the brain region analysed and that this transcript is brain specific. Secondly, Williamson et al. (2012) found that the major CDKL5 mRNA transcript expressed in the brain contained only 18 exons. Exon 18 was shown to be extended by 170 bases including a termination codon. The authors demonstrated that this novel 107kDa (CDKL5107) protein and CDKL5115 had the same functional properties (Williamson et al. 2012). Williamson et al. (2012) were unable to identify the 3' untranslated region (3' UTR) of the 107kDa variant but

Montini et al (1998) showed, by Northern blotting, that the size of the brain-expressed CDKL5 transcript was approximately 9500 bases in both human and mouse whereas in the mouse testis an abundant ~3500 base transcript was found. This suggests that the CDKL5 reference sequence (~3400 bases) represents the testis-specific isoform and that the full-length, brain-expressed transcript remains to be identified.

The study by Williamson et al. (2012) suggests that the only tissue that expresses CDKL5 isoform II and CDKL5115 is the testis. They demonstrated that all other tissues, including fetal brain, express CDKL5 isoform I and CDKL5107. Because CDKL5107 consists of only 18 exons it has been suggested that screening for functional mutations in exons 19-21 of the CDKL5 gene may not be useful (Diebold et al. 2013). To date, no proven disease causing mutations have been identified in exons 19-21.

The expression of CDKL5 in the developing mouse brain is low at embryonic day 16.5 (E16.5) but from then on until postnatal day 14 (P14) expression increases and then slowly declines during postnatal life. Expression of CDKL5 is particularly high in the forebrain relative to other brain areas (Wang et al. 2012). The presence of a CpG island overlapping exon 1 of the CDKL5 gene has suggested that its expression may in part be mediated by MECP2. One recent study (Carouge et al. 2010) has shown that, in the rat, reducing MECP2 levels using siRNA causes an increase in CDKL5 expression. However, other studies have shown that CDKL5 expression is unaltered in lymphoblastoid cell lines from patients with classical RTT (Mari et al. 2005) and in the brains of MECP2 deficient mice (Weaving et al. 2004) suggesting that no interaction between CDKL5 and MECP2 occurs at the level of transcription.

Function

CDKL5 is a serine/threonine kinase of the CMGC kinase family. It is characterised by a catalytic domain (amino acids 13-297) which contains an ATP-binding region (amino acids 13-43), the serine/threonine protein kinase active site (amino acids 131-143) and a Thr – X – Tyr (TEY) motif at amino acids 169-171. Putative signals for nuclear import and export are located in the C-terminal domain of the protein. It has the ability to phosphorylate a number of proteins and importantly, to auto-phosphorylate its TEY motif.

Localisation

The function of CDKL5 seems, in part, to be regulated both through its subcellular localization and its synthesis and degradation. In the brain, mouse CDKL5 is found mainly in the cytoplasmic compartment but after P14 it starts to accumulate in the nucleus, where approximately 40% of total CDKL5 can be detected. However, this translocation only seems to occur in certain brain areas: in the cerebellum approximately 80% of CDKL5 remains cytoplasmic but in the cortex CDKL5 is equally distributed between the cytoplasmic and nuclear compartments (Rusconi et al. 2008). Exogenous CDKL5 shuttles between the nuclear and cytoplasmic compartments in cultured non-neuronal cells; this involves the C-terminus of the protein and the CRM1 nuclear export receptor. This mechanism may not apply in neuronal cells, however, because in resting hippocampal neurons, the translocation of mouse CDKL5 from the nucleus to the cytoplasm seems to be regulated by specific

stimuli.

For example, glutamate treatment induces nuclear export of CDKL5 leading to its accumulation in the cytoplasm (Rusconi et al. 2011). Williamson et al. (2012) demonstrated that the CDKL5107 isoform had a similar subcellular localisation to that of CDKL5115 but it is unclear whether the CDKL5-E16b isoform has a similar localisation pattern.

Cell-based models

The location of CDKL5 within the cell seems to be important for its function. CDKL5 has the ability to phosphorylate itself and, in the nucleus, it is able to phosphorylate DNA methyl transferase I (DNMTI; Kameshita et al. 2008) and there is also some evidence that it is able to phosphorylate MECP2 in vitro (Mari et al. 2005; Bertani et al. 2006; Williamson et al. 2012). In addition, the kinase activity of CDKL5 is associated with a number of RNA splicing factors that are stored within nuclear "speckles" (Ricciardi et al. 2009) where CDKL5 may be important in the phosphorylation of the RS domain of serine-rich (SR) splicing factors. Phosphorylation of these splicing factors is necessary for their release from nuclear speckles and directs them to sites where pre-mRNA splicing occurs. Ricciardi et al. (2009) have suggested that CDKL5 acts on nuclear speckle disassembly and the subsequent redistribution of some of the speckle-associated splicing factors. These results suggest that CDKL5 may play a role in controlling gene expression through phosphorylation of DNMTI and alteration of CpG methylation which may affect the transcription of numerous genes. In addition, altering the distribution of the splicing factor machinery within the nucleus may result in alternative splicing of different RNA's leading to a subset of proteins with subtly altered functions. Rosas Vargas et al. (2008) looked at the effect on cellular distribution of two missense mutations (p.Ala40Val and p.Leu220Pro), which are associated with severe infantile encephalopathy. These mutations are both located within the catalytic domain of CDKL5 and are therefore likely to affect phosphorylation. In both cases the proteins were unable to cross into the nucleus suggesting that the phosphorylation state of CDKL5 may regulate its ability to enter the nucleus.

In the cytoplasm CDKL5 also appears to have a range of functions. CDKL5 affects neuronal morphogenesis and dendritic arborisation through rearrangement of the cytoskeleton. It co-localises with F-actin and interacts with Rac-1, a Rho GTPase, which promotes the formation and/or maturation of spines by remodelling the actin cytoskeleton (Chen et al. 2010). CDKL5 is thought to act upstream of Rac -1 to influence neuronal morphogenesis. Brain-derived neurotrophic factor (BDNF) which transiently phosphorylates CDKL5, is also necessary for Rac-1 activation. In the absence of CDKL5, Rac-1 is not activated by BDNF (Chen et al. 2010). Ricciardi et al. (2012) demonstrated that CDKL5 localised almost exclusively at the post synaptic density (PSD) of excitatory synapses both in vivo and in vitro. In addition the same group demonstrated that in iPSC-derived neurons from patients with CDKL5 mutations exhibit aberrant dendritic spines. Depletion of CDKL5 in rat hippocampal neurons indicated that CDKL5 is required for ensuring a correct number of well-shaped spines. These morphological alterations were associated with a reduction in the number of excitatory synapses and a significant decrease in spontaneous miniature excitatory postsynaptic currents (mEPSCs). There was no significant effect on inhibitory synapse density or any significant changes in miniature inhibitory

postsynaptic currents (Riciardi et al. 2012). It was shown that CDKL5 interacted with and phosphorylates the Netrin G1 receptor (NGL-1), a synaptic cell adhesion molecule which plays a crucial role in early synapse formation and subsequent maturation. NGL-1 binds to PSD95, a protein that plays a significant role in learning and memory, and this binding is stabilised by phosphorylation of NGL-1 at Ser631 by CDKL5. This stabilisation of the NGL-1/PSD95 interaction ensures that PSD95 is targeted to new forming dendritic protrusions (Riciardi et al. 2012). CDKL5 also binds directly to PSD95 (Zhu et al. 2013; Zhang et al. 2014). When PSD95 is palmitoylated at its amino terminal CDKL5 is able to bind to it which promotes targeting of CDKL5 to excitatory synapses (Zhu et al. 2013). PSD95 palmitoylation is dynamic and reversible: de-palmitoylation is accelerated by glutamate receptor activation whereas palmitoylation is increased by blocking synaptic activity (El-Husseini et al. 2002; Noritake et al. 2009; Yoshii et al. 2011). Palmitoylated PSD95 associates with the synaptic membrane and CDKL5 and serves as a "slot" for AMPA type glutamate receptor (AMPAR) through the interaction with TARP/Stargazin. When stimulated by glutamate, $Ca2+$ influx through the NMDA receptor occurs, which induces binding of $Ca2+$/calmodulin to PSD95 blocking palmitoylation and facilitating de-palmitoylation of PSD95 (Zhang et al. 2014). This leads to dissociation of PSD95 from both the synaptic membrane and CDKL5 leading to a reduction in the number of AMPAR "slots" and a reduction in AMPAR mediated synaptic transmission (Zhang et al. 2014). Neuronal depolarization using potassium chloride induces a rapid increase of CDKL5 protein levels, mostly mediated by translation outside the soma in the dendrites (La Montanara et al. 2015). In young neurons this induction is prolonged but in mature neurons the induction is transient, returning to basal levels within ten minutes. Whether this difference in induction is because mature synapses are not present in immature neuronal cultures compared to mature neuronal cultures is unclear. It was shown that $Ca2+$ influx is necessary for CDKL5 protein synthesis which in turn induces protein phosphatase-1 (PP1) dependent dephosphorylation of CDKL5 and its degradation. PP1 is activated by NMDA receptor stimulation by inhibiting its phosphorylation by CDK5 (Hou et al. 2013). Another regulator of CDKL5 activity is the ubiquitin ligase Mind Bomb 1 (Mib1) which ubiquinates CDKL5 and alters its localization, abundance and functional effect (Mertz 2015). Mib1 has been shown to regulate neurite outgrowth (Choe et al. 2007) and synaptic plasticity (Yoon et al. 2012) but it is unclear whether Mib1 has a role in CDKL5 regulation upon synaptic activation.

This data implies that at least two pathways are important in mediating the synaptic function of CDKL5: CDKL5 in conjunction with Rac-1 and BDNF forming one pathway and a second containing CDKL5, PSD95 and NGL-1 complex. It is possible that interaction between these two pathways is mediated by Kalirin-7. Kalirin-7 is a Rho guanine nucleotide exchange factor (GEF) which promotes the exchange of GDP for GTP thus stimulating the activity of specific Rho GTPases, in this case Rac-1 (Mandela and Ma 2012). Kalirin-7 is associated almost exclusively with post-synaptic excitatory terminals where it interacts with PSD95. In Kalirin-7 deficient mice there is 15% decrease in spine density and PSD's isolated from these mice show a deficit in Cdk5, a kinase known to phosphorylate Kalirin-7 and play an essential role in Kalirin-7-mediated spine formation and synaptic function (Ma et al. 2008). Cyclin dependent kinase 5 (CDK5) is another member of the CMGC kinase family and it would be of

interest to know if CDKL5 is also able to phosphorylate Kalirin-7.

An identified substrate for phosphorylation by CDKL5 has been identified as amphiphysin 1(AMPH1) which is a multifunctional adaptor molecule involved in neurotransmission and synaptic vesicle recycling through clathrin-mediated endocytosis (Sekiguchi et al 2013; Katayama et al. 2015). CDKL5 phosphorylates AMPH1 exclusively at Ser 293 and this phosphorylation is disrupted by CDKL5 catalytic domain mutations. Interestingly CDK5 also phosphorylates AMPH1 but at different positions (Ser-272, 276 and 285) to that of CDKL5 (Sekiguchi et al 2013). It remains unclear what effect phosphorylation of AMPH1 by CDKL5 has on its function but the Amph1 deficient mouse shows major learning difficulties and seizures that were unresponsive to treatment, suggesting it plays a role in neural development and transmission (Di Paolo et al.2002).

A recent study found that GRID1 encoding the Glutamate D1 receptor (GluD1) was up-regulated in both MECP2- and CDKL5- mutated human iPS cells (Livide et al. 2015). GluD1 is widely expressed in the brain (Hepp et al. 2015) and knockout GluD1 mice have been shown to have a higher number of dendritic spines, greater excitatory neurotransmission and a higher number of synapses in the medial prefrontal cortex (Gupta et al. 2015). An increase in GluD1 protein levels may well cause a decrease in dendritic spine numbers and excitatory synapses and altered neurotransmission as was shown by Riciardi et al. (2012).

CDKL5 has been shown to be up-regulated when neuroblastoma cell lines are differentiated in the presence of retinoic acid (Valli et al. 2012). This up-regulation has two effects in the SH-SY5Y neuroblastoma cell line: promotion of neuronal differentiation and reduction of cell proliferation by blocking cell cycle progression. It was also shown that MYCN, a transcription factor belonging to the MYC family, negatively regulates CDKL5 (Valli et al. 2012). MYCN is primarily expressed in normal developing embryos and is thought to be critical in brain and other neural development promoting rapid cell division. Valli et al. (2012) suggest that the transition from embryonic to postnatal brain development, leads to reduced MYCN expression and increased CDKL5 expression, resulting in a shift from cell proliferation to differentiation.

From these studies it suggests that CDKL5 plays a major role in synaptic function, although the precise role(s) remains to be elucidated. To date, there is very little information on what targets CDKL5 phosphorylates within the synapse, which will be important in understanding its function. It also seems that CDKL5 may play an important role in proliferation and development of neuronal cells, although this has only been shown in one study using a neuroblastoma cell line.

Mouse models of CDKL5 deficiency

Two different mouse knockout models of CDKL5 deficiency have been used to investigate both phenotypic and functional aspects of CDKL5 loss. Wang et al. (2012) generated a mouse based on a patient-specific mutation that results in skipping of exon 6 of mouse CDKL5 and results in a premature termination codon in the N-terminal kinase domain. This mouse shows the majority of the symptoms shown by human patients with the major exception that these mice do not have spontaneous seizures. This may be due to the strain of mouse used in this study or reflect a distinct functional difference between the human and mouse homologues of CDKL5.

Wang et al. (2012) used this model to examine the effect of a loss of CDKL5 on kinase activity. They found that there was a marked decrease in activity for the adenosine monophosphate-activated protein kinase (AMPK), protein kinase A (PKA) and AKT substrates in the forebrain of male CDKL5-/y mice. Other substrates were also affected including those for mitogen-activated protein kinase (MAPK), ataxia-telangiectasia mutated/ ataxiatelangiectasia and Rad3-related (ATM/ATR), and cyclin-dependent kinase (CDK) substrates (Wang et al. 2012). Further evaluation of the AKT pathway showed that both phosphorylation of AKT at Ser473 and the mechanistic Target of Rapamycin (mTOR) at Ser 2448 were reduced despite normal levels of total AKT and mTOR protein (Wang et al. 2012).

A second Cdkl5 knockout mouse was generated by germ line deletion of exon 4 of the CDKL5 gene (Amendola et al. 2014). Behavioural deficits were noted in both homozygous female and hemizygous male CDKL5 knockout mice which included abnormal hind limb clasping, hypolocomotion and decreased head tracking compared to wild-type litter mates (Amendola et al. 2014). These mice also showed reduced dendritic aborization and decreased phosphorylation of AKT and ribosomal protein S6 (rpS6). Amendola et al. (2014) also independently and specifically knocked out Cdkl5 from GABAergic and glutamatergic neurons and studied the behavioural effects. They found that defects in hind limb clasping and head tracking were associated with CDKL5 knock-out in glutamatergic neurons whereas defects in hypolocomotion were associated with this knock-out in GABAergic neurons, suggesting that different behavioural characteristics can be localised to different forebrain neuronal populations (Amendola et al. 2014). As with the model of Wang et al. (2012), no evidence of spontaneous seizures was found both in the constitutive and the conditional knockout mice and this was also not strain specific. However, administration of kainic acid to both CDKL5 deficient and wild type mice did show altered EEG burst activity in deficient mice which may be useful in understanding the epileptic mechanism found in human CDKL5 deficiency (Amendola et al. 2014).

Using the same CDKL5 mouse model as Amendola et al. (2014), Fuchs et al (2014) studied neuronal development in the hippocampal dentate gyrus which occurs predominantly in the postnatal period. They found that CDKL5 deficiency caused increased proliferation of intermediate progenitor cells (IPCs) but also an increase in apoptosis of immature neurons derived from IPCs suggesting that Cdkl5 promotes the survival and maturation of postmitotic neurons (Fuchs et al. 2014). Signalling through AKT/GSK-3β was also shown to be disrupted. A decrease in GSK-3β at serine 9 was found, which leads to increased GSK-3β activity. When Cdkl5 was re-expressed both AKT/GSK-3β signalling and neuronal survival were restored suggesting that CDKL5 plays a key role in neurogenesis and dendritic development in the hippocampal dentate gyrus (Fuchs et al. 2014). More recently Fuchs et al. (2015) have shown that an inhibitor of GSK-3β, SB216763, is able to restore neuronal precursor survival and maturation in the hippocampus in hemizygous male Cdkl5 knockout mice. Hippocampal dependent memory tests were also improved by SB216763 treatment, whereupon they become similar to those of wild type mice (Fuchs et al. 2015). These data suggest that GSK-3β inhibition may be of therapeutic benefit in CDKL5 disorder but it would be useful to determine the therapeutic effect of SB216763 on heterozygous CDKL5 deficient female mice in the first instance. It would also be interesting to know whether inhibition of GSK-3β restored AKT and

rpS6 phosphorylation to normal levels.

From these mouse studies CDKL5 appears to play a role in both the regulation of neurogenesis and neural maturation affecting both the AKT/GSK-3β (Fuchs et al. 2014) and mTOR pathways (Wang et al. 2012; Amendola et al. 2014). mTOR is the catalytic subunit of two distinct complexes, termed mTOR complex 1 (mTORC1) and mTORC2, which are distinguished from each other by unique accessory proteins that function as scaffolds allowing the binding of other regulators and defining their substrate specificity (reviewed by Zoncu et al. 2011). The mTOR and AKT pathways interact: mTORC2 is known to phosphorylate AKT at Ser473 which in turn, when phosphorylated, is able to phosphorylate mTOR leading to mTORC1 activation and phosphorylation of rpS6. This implies that defects in either one of these pathways will impact on the other, which is seen in both of the Cdkl5 deficient mouse models.

mTORC1 and mTORC2 activity are important in neuronal development, playing a role in axon guidance, dendrite development and dendritic spine morphogenesis (Campbell and Holt 2001; Jaworski et al. 2005; Kumar et al. 2005; Tavazoie et al. 2005; Urbanska et al. 2012; Lipton and Sahin 2014). mTORC1 is also essential for several forms of synaptic plasticity that underlie processes of learning and memory formation (Cammalleri et al. 2003; Hou and Klann 2004; Parsons et al. 2006; Tang et al. 2002; Tischmeyer et al. 2003). mTORC2 is thought to be involved in actin dynamics via the small GTPase Rho family which includes Rac-1 (Jacinto et al. 2004) and are known to be positive regulators of dendritic growth (Murakoshi et al. 2011; Woo and Gomez 2006). Saci et al. (2011) demonstrated that Rac-1 expression, but not its GTP binding activity, regulates both the mTORC1 and mTORC2 complexes. They demonstrated that the carboxy terminal of Rac-1 is important in determining mTOR localization in the perinuclear region and plasma membrane (Saci et al. 2011). mTOR and Rac-1 have also been shown to be necessary for axonal branching (Grider et al. 2009) and in controlling dendritic spine density in response to hormone treatment (Lee et al. 2011). These results suggest that CDKL5 may play a role in regulating mTOR activity through its interaction with Rac-1 at the synapse.

It is worth noting that a study on the male Mecp2tml.lJae mouse RTT model (Chen et al. 2001) showed a similar decrease in AKT, mTOR and rpS6 phosphorylation to that found in the CDKL5 knock-out mice (Ricciardi et al. 2011) which suggests that CDKL5 and MECP2 deficiency may affect similar pathways, including the mTOR pathway. Fuchs et al. (2014) also recognised that similar pathways were affected but noted that in MECP2 deficient mice there is no evidence for altered neuronal proliferation or survival. They also suggested that dendritic hypertrophy may explain the common features seen between RTT and CDKL5 disorder in humans (Fuchs et al. 2014). It remains to be seen whether GSK-3β phosphorylation is altered in MECP2 deficient mice.

Restoring GSK-3β function in CDKL5 deficient mice using SB216763 was sufficient to correct the cognitive defects seen in these mice (Fuchs et al. 2015). This implies that although multiple signalling pathways are dysregulated in the CDKL5 deficient mouse brain (Wang et al. 2012), restoring the function of just one pathway seems able to restore function to near normal. It will be important to see if the other deficits found in human CDKL5 disorder are restored to normal using GSK-3β inhibitors.

Conclusion

Deficiency of the protein CDKL5 causes severe learning difficulties and seizures. CDKL5 has been shown to be a multifunctional protein which has multiple effects within the neuronal cell. One of its main functions appears to be in neuronal development and synaptic structure and activity in particular. This has led to the possibility of a potential target for therapeutic intervention in CDKL5 disorder.

This review was been written for CDKL5 UK in July 2013 and updated August 2015. It is the intellectual property of CDKL5 UK and its author David Millar, Lecturer in Human Molecular Genetics, Institute of Medical Genetics, School of Medicine, Cardiff University. Requests to reproduce this article should be addressed to CDKL5 UK via carolanne@cdkl5uk.org

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