

## 論文の内容の要旨

### Characterization of *Dclk1* isoforms in the developing mouse brain

(マウス大脳皮質発生における *Dclk1* スプライシングアイソフォームの解析)

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## Background

*DCLK1* is a neuronal serine-threonine protein kinase belonging to the Doublecortin (*DCX*) protein family, which in turn was originally identified as a causative gene for human cortical malformation. *DCLK1* is involved in multiple brain development processes including cell migration, axon/dendrite growth, and synapse development (Shu et al. 2006; Koizumi et al., 2006; Dauel et al., 2006; Shin et al., 2013). The genetics of *DCLK1* are quite complex, and in mouse the *Dclk1* 20 exons give rise to multiple splicing variants through alternative promoters, but the detailed expression patterns in the brain as well as *in vivo* functions of each isoform are remains incompletely understood. To date, 12 variants have been discovered, grouped into four groups: the long DCLK1-L, which contains both the microtubule-binding domains and the kinase domain, a variant with only the microtubule binding domains (DCL), a short isoform which only carries the kinase domain (CPG16), and an even shorter variant, CARP. DCLK1-L and DCL function together with DCX in neuronal migration and axon elongation (Koizumi et al., 2006). DCLK1-L expression is prominent in the embryonic brain, gradually declines during the development, and remains in the adulthood (Shin et al., 2013; Koizumi et al., 2017; Zygmunt et al., 2018). In cultured hippocampal neurons, DCLK1-L is localized at neurite tips and in the soma, and promotes dendrite growth and branching (Burgess and Reiner, 2000; Lin et al., 2000; Shin et al., 2013; Lipka et al., 2016). DCL likely acts together with DCLK1-L and DCX in the embryonic stage (Koizumi et al., 2006; Deuel et al., 2006), but, unlike DCLK1-L and DCX, histochemical studies have indicated that DCL expression declines during the late embryonic stages (E12-17), and in adult becomes confined to the neurogenic regions (Boekhoorn et al., 2008; Saaltink et al., 2012). On the other hand, CPG16 and CARP have been less studied, and their functions remain largely unknown. CPG16 was originally identified as being upregulated by the kainite-induced long-term potentiation (LTP) in the hippocampus, and previous studies suggested potential roles for CPG16 and CARP in the down-regulation of cascades downstream cAMP and in memory consolidation, respectively. Recently, thanks to genome wide association studies and transcriptome analyses, *DCLK1* has been identified as one of the causative genes in both higher cognitive functions and several neurodevelopmental disorders (Le Hellard et al., 2009; Havik et al., 2012; Wu et al., 2012). Despite their importance in physiology and pathology, the differential expression pattern of these variants as well as *in vivo* functions is still unclear, and therefore is the focus of this study.

## Results

I assessed the expression patterns of DCLK1 isoforms using multiple platforms including *in silico*, *in situ*, and *in vitro* datasets, and provided compelling evidence that DCLK1 isoforms are distributed in the partially distinct brain regions in both developmental and adult stages. First, I focused on the cortex and performed Western Blots (WBs) at different time points, namely E14.5, P0, P7, P15, and P30. Using two different antibodies, binding the N-terminus MT-binding domain and the C-terminus of the kinase domain, respectively, I identified the distinct temporal expression pattern of the isoforms, with DCL being rapidly downregulated postnatally, stable expression of DCLK1-L, which slowly wanes postnatally, and rapid upregulation of CPG16 from P7. I also investigated these patterns of expression further by using publicly available RNA-Seq data to achieve Sashimi plots, which are a graphical representation of transcriptome data from which information circa alternative splicing can be extracted. Via my *in silico* analysis, I was able to locate signature junctions which are necessary for determining the isoform-specific expression. Comparing these datapoints together with the Sashimi plots, which map read depth on each exon as an histogram, I was able to determine that DCLK1-L is the major isoform type in the prenatal development because of its stable and high level of expression and CPG16 becomes the most abundant one in the postnatal maturation. DCL and CARP may have minor contributions relative to the other two. This is of particular interest as *in silico* analysis has not yet been applied to study developmental dynamics of the alternatively spliced transcripts of *Dclk1*.

Next, I carried out In situ Hybridization (ISH) experiments to characterize the spatiotemporal pattern of mRNA expression of the different isoforms. Due to the absence of a single exon specifically defining a given isoform, each probe is designed to recognize an exon shared by a pair of isoforms. Nonetheless, it is possible to infer information about the expression patterns of each single isoform by comparing the ISH results in pairs. Looking at P7 and P30 in the cortex, expression of DCLK1-L is greater in the upper layers of the cortex, and is decreased by P30. On the other hand, CPG16 shows an opposite pattern of expression, with predominant expression at P30, and a very different localization, as it appears to be evenly distributed across the cortex. In addition, spatially segregated expression of DCLK1 variants can be observed in the hippocampus. Using the same comparison principle, I could determine that DCLK1-L showed a ubiquitous signal in all regions of hippocampus, whereas CPG16 signals were observed in the Ammon's horn (CA1-3), whereas DCL signals were mainly localized in the dentate gyrus (DG) and CA1.

I also conducted a further *in vivo* and *in vitro* study by in utero electroporation as well as dissociated cortical primary culture transfected with vectors expressing each isoform. I further showed that overexpression of DCLK1-L, but no other isoforms, in immature cortical neurons causes severe migration defects in the cortex and that the migration defects are dependent on the kinase activity of DCLK1-L. In primary culture, DCLK1-L both induced increased neurite length and complexity, with other isoforms having a minor effect. This neuronal phenotypes induced by DCLK1-L overexpression are likely to be dependent on the kinase activity of DCLK1-L, as no obvious migration defects in cortical neurons nor branching phenotypes in cultured neurons were observed by overexpressing the kinase-dead version of DCLK1-L.

## Conclusions and Perspectives

In summary, in this study I dissected the *in vivo* expression patterns of *Dclk1* isoforms in different developmental stages of the mouse brain using multiple data platforms. Additionally, I also provided the evidence that DCLK1-L overexpression impacts the radial migration of developing cortical neurons in a kinase activity dependent manner. Given that a variety of data platforms including deep sequencing data and RNAseq data in the brain have become available, further studies using multiple data platforms will help to elucidate isoform-specific expression and function in the developing and mature brain.