

## カルパイン阻害剤を用いた滑脳症治療への新戦略

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### **A novel strategy for therapeutic intervention to lissencephaly using calpain inhibitors**

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1. Pramparo T, Libiger O, Jain S, Li H, Youn YH, Hirotsune S, Schork NJ, Wynshaw-Boris A. (2011)

#### **Global developmental gene expression and pathway analysis of normal brain development and mouse models of human neuronal migration defects.**

PLoS Genet, 7: e1001331.

Heterozygous LIS1 mutations are the most common cause of human lissencephaly, a human neuronal migration defect, and DCX mutations are the most common cause of X-linked lissencephaly. LIS1 is part of a protein complex including NDEL1 and 14-3-3ε that regulates dynein motor function and microtubule dynamics, while DCX stabilizes microtubules and cooperates with LIS1 during neuronal migration and neurogenesis. Targeted gene mutations of Lis1, Dcx, Ywhae (coding for 14-3-3ε), and Ndel1 lead to neuronal migration defects in mouse and provide models of human lissencephaly, as well as aid the study of related neuro-developmental diseases. Here we investigated the developing brain of these four mutants and wild-type mice using expression microarrays, bioinformatic analyses, and in vivo/in vitro experiments to address whether mutations in different members of the LIS1 neuronal migration complex lead to similar and/or distinct global gene expression alterations. Consistent with the overall successful development of the mutant brains, unsupervised clustering and co-expression analysis suggested that cell cycle and synaptogenesis genes are similarly expressed and co-regulated in WT and mutant brains in a time-dependent fashion. By contrast, focused co-expression analysis in the Lis1 and Ndel1 mutants uncovered substantial differences in the correlation among pathways. Differential expression analysis revealed that cell cycle, cell adhesion, and cytoskeleton organization pathways are commonly altered in all mutants, while synaptogenesis, cell morphology, and inflammation/immune response are specifically altered in one or more mutants. We found several commonly dysregulated genes located within pathogenic deletion/duplication regions, which represent novel candidates of human mental retardation and neurocognitive disabilities. Our analysis suggests that gene expression and pathway analysis in mouse models of a similar disorder or within a common pathway can be used to define novel candidates for related human diseases.

2. Torisawa T, Nakayama A, Furuta K, Yamada M, Hirotsune S, Toyoshima YY. (2011)

#### **Functional dissection of LIS1 and NDEL1 towards understanding the molecular mechanisms of cytoplasmic dynein regulation.**

J Biol Chem, 286: 1959-1965.

LIS1 and NDEL1 are known to be essential for the activity of cytoplasmic dynein in living cells. We previously reported that LIS1 and NDEL1 directly regulated the motility of cytoplasmic dynein in an in vitro motility assay. LIS1 suppressed dynein motility and inhibited the translocation of microtubules (MTs), while NDEL1 dissociated dynein from MTs and restored dynein motility following suppression by LIS1. However, the molecular mechanisms and detailed interactions of dynein, LIS1, and NDEL1 remain unknown. In this study, we dissected the regulatory effects of LIS1 and NDEL1 on dynein motility using full-length or truncated recombinant fragments of LIS1 or NDEL1. The C-terminal fragment of NDEL1 dissociated dynein from MTs, whereas its N-terminal fragment restored dynein motility following suppression by LIS1, demonstrating that the two functions of NDEL1 localize to different parts of the NDEL1 molecule, and that restoration from LIS1 suppression is caused by the binding of NDEL1 to LIS1, rather than to dynein. The truncated monomeric form of LIS1 had little effect on dynein motility, but an artificial dimer of truncated LIS1 suppressed dynein motility, which was restored by the N-terminal fragment of NDEL1. This suggests that LIS1 dimerization is essential for its regulatory function. These results shed light on the molecular interactions between dynein, LIS1, and NDEL1, and the mechanisms of cytoplasmic dynein regulation.

3. Yamada M, Toba S, Takitoh T, Yoshida Y, Mori D, Nakamura T, Iwane AH, Yanagida T, Imai H, Yu-Lee LY, Schroer T, Wynshaw-Boris A, Hirotsune S. (2010)

### **mNUDC is required for plus-end-directed transport of cytoplasmic dynein and dynactins by kinesin-1.**

EMBO J, 29: 517-531.

Lissencephaly is a devastating neurological disorder caused by defective neuronal migration. The LIS1 (or PAFAH1B1) gene was identified as the gene mutated in lissencephaly patients, and was found to regulate cytoplasmic dynein function and localization. In particular, LIS1 is essential for anterograde transport of cytoplasmic dynein as a part of the cytoplasmic dynein-LIS1-microtubule complex in a kinesin-1-dependent manner. However, the underlying mechanism by which a cytoplasmic dynein-LIS1-microtubule complex binds kinesin-1 is unknown. Here, we report that mNUDC (mammalian NUDC) interacts with kinesin-1 and is required for the anterograde transport of a cytoplasmic dynein complex by kinesin-1. mNUDC is also required for anterograde transport of a dynactin-containing complex. Inhibition of mNUDC severely suppressed anterograde transport of distinct cytoplasmic dynein and dynactin complexes, whereas motility of kinesin-1 remained intact. Reconstruction experiments clearly demonstrated that mNUDC mediates the interaction of the dynein or dynactin complex with kinesin-1 and supports their transport by kinesin-1. Our findings have uncovered an essential role of mNUDC for anterograde transport of dynein and dynactin by kinesin-1.

4. Yamada M, Yoshida Y, Mori D, Takitoh T, Kengaku M, Umeshima H, Takao K, Miyakawa T, Sato M, Sorimachi H, Wynshaw-Boris A, Hirotsune S. (2009)

### **Inhibition of calpain increases LIS1 expression and partially rescues in vivo phenotypes in a mouse model of lissencephaly.**

Nat Med, 15: 1202-1207.

Lissencephaly is a devastating neurological disorder caused by defective neuronal migration. LIS1 (official symbol PAFAH1B1, for platelet-activating factor acetylhydrolase, isoform 1b, subunit 1) was identified as the gene mutated in individuals with lissencephaly, and it was found to regulate cytoplasmic dynein function and localization. Here we show that inhibition or knockdown of calpains protects LIS1 from proteolysis, resulting in the augmentation of LIS1 amounts in Lis1(+/-) mouse embryonic fibroblast cells and rescue of the aberrant distribution of cytoplasmic dynein, mitochondria and beta-COP-positive vesicles. We also show that calpain inhibitors improve neuronal migration of Lis1(+/-) cerebellar granular neurons. Intraperitoneal injection of the calpain inhibitor ALLN to pregnant Lis1(+/-) dams rescued apoptotic neuronal cell death and neuronal migration defects in Lis1(+/-) offspring. Furthermore, in utero knockdown of calpain by short hairpin RNA rescued defective cortical layering in Lis1(+/-) mice. Thus, calpain inhibition is a potential therapeutic intervention for lissencephaly.

5. Mori D, Yamada M, Mimori-Kiyosue Y, Shirai Y, Suzuki A, Ohno S, Saya H, Wynshaw-Boris A, Hirotsune S. (2009)

### **An essential role of the aPKC-Aurora A-NDEL1 pathway in neurite elongation by modulation of microtubule dynamics.**

Nat Cell Biol, 11: 1057-1068.

Orchestrated remodelling of the cytoskeleton is prominent during neurite extension. In contrast with the extensive characterization of actin filament regulation, little is known about the dynamics of microtubules during neurite extension. Here we identify an atypical protein kinase C (aPKC)-Aurora A-NDEL1 pathway that is crucial for the regulation of microtubule organization during neurite extension. aPKC phosphorylates Aurora A at Thr 287 (T287), which augments interaction with TPX2 and facilitates activation of Aurora A at the neurite hillock, followed by phosphorylation of NDEL1 at S251 and recruitment. Suppression of aPKC, Aurora A or TPX2, or disruption of Ndel1, results in severe impairment of neurite extension. Analysis of microtubule dynamics with a microtubule plus-end marker revealed that suppression of the aPKC-Aurora A-NDEL1 pathway resulted in a significant decrease in the frequency of microtubule emanation from the microtubule organizing centre (MTOC), suggesting that Aurora A acts downstream of aPKC. These findings demonstrate a surprising role of aPKC-Aurora A-NDEL1 pathway in microtubule remodelling during neurite extension.