De novo variants in RHOBTB2, an atypical Rho GTPase gene, cause epileptic encephalopathy

博士（医学）　Md. Hazrat Belal

論文題目

De novo variants in *RHOBTB2*, an atypical Rho GTPase gene, cause epileptic encephalopathy

（非定型 Rho GTPase をコードする *RHOBTB2* の de novo 変異はてんかん性脳症を引き起こす）

論文の内容の要旨

[Introduction]

Epileptic encephalopathies (EEs) are severe neurological disorders characterized by frequent seizures, cognitive and motor impairments. Several mutations in novel causative genes have been identified as the major genetic cause of EEs. One such gene is RHOBTB2, which encodes Rho-related BTB domain-containing protein 2 (RHOBTB2), an atypical Rho GTPase. RHOBTB2 is a substrate-specific adaptor for the Cullin-3 (CUL3) based ubiquitin ligase complex that recruits target proteins for degradation. RHOBTB2 itself is a substrate for the CUL3 complex. In this study, we have identified three de novo RHOBTB2 variants in three patients with EE and confirming that de novo RHOBTB2 variants are a genetic cause for EE.

[Patients and Methods]

Patients and whole exome sequencing (WES): After obtaining written informed consent, WES was performed for a total of 1,230 patients with infantile or early childhood-onset epilepsy using genomic DNA extracted from blood leukocytes. Exonic DNA fragments were captured by SureSelectXT Human All Exon v4, v5, or v6 kits and were sequenced using Illumina Hiseq 2000 or 2500 systems. Detailed clinical information was obtained from corresponding clinicians. Experimental protocols were approved by the institutional review board of Yokohama City University School of Medicine (A140925001), Showa University School of Medicine (No. 219), and Hamamatsu University School of Medicine (17-163).

Expression vectors and functional analysis: Site directed mutagenesis was used to generate RHOBTB2 mutants from human RHOBTB2 cDNA clone (FXC00611). All variant cDNAs were cloned into the pCAGGS-IRES2-nucEGFP vector to express FLAG-tagged wild-type (WT) or mutant RHOBTB2. RHOBTB2 expressing vectors were transfected into Neuro-2a (N2a) cells by electroporation to check RHOBTB2 stability by immunoblotting (IB) using anti FLAG antibody. In addition, co-immunoprecipitation (co-IP) of transiently co-expressed FLAG-RHOBTB2 and HA-CUL3 was performed using anti HA antibody to examine the binding of RHOBTB2 WT/mutant to CUL3.

[Results]

Using the trio-based WES data of 337 families, we found two de novo variants in RHOBTB2 (NM_001160036.1), c.1448G>A, p.(Arg483His), and c.1532G>A, p.(Arg511Gln) from two unrelated Japanese families. Analyzing WES data from 893 cases, we identified a novel RHOBTB2 variant, c.1519C>T, p.(Arg507Cys) in one patient. All variants substitute amino acids that are evolutionarily conserved among vertebrates, and are clustered between the first and second BTB domain, and Arg483 (n = 5) and Arg511 (n = 5) residues are recurrently affected. All three patients showed acute encephalopathy (febrile status epilepticus), with magnetic resonance imaging revealing hemisphere swelling or reduced diffusion in various brain regions. Infantile onset seizures, severe intellectual disabilities, impaired motor functions and postnatal microcephaly were also observed in at least two of the three patients.

To examine the mutational effect of three missense variants, WT RHOBTB2 and three mutants were transiently expressed in N2a cells. The level of WT RHOBTB2 was very low, while MG132 (a proteasome inhibitor) treatment increased the amount of WT RHOBTB2, indicating that RHOBTB2 suffered from proteasomal degradation. Interestingly, the levels of three mutant RHOBTB2 without MG132 treatment were higher than that of WT RHOBTB2. In addition, the RHOBTB2 WT level was reduced by co-expression of CUL3, whereas little or no changes were observed in the level of mutant RHOBTB2. Co-IP assay to examine binding between CUL3 and RHOBTB2 revealed no differences between CUL3 and each of the three mutants and WT RHOBTB2. These data indicate that impaired degradation of the three mutants by the CUL3 complex is not mediated by decreased binding with CUL3.

[Discussion]

Consistent with a previous report of 10 patients with de novo RHOBTB2 variants, febrile status epilepticus was observed in all three patients in our study, indicating that acute encephalopathy might be a relatively specific feature in RHOBTB2 related EEs.

The first BTB domain of RHOBTB2 is essential for binding with CUL3. Therefore, it is reasonable that we did not find any impaired binding between any of the three mutant and CUL3, because these variants do not involve the first BTB domain. Nonetheless, transient expression experiments in N2a cells showed that the three mutants tended to be more abundantly expressed than WT, and co-transfection of CUL3 reduced the level of WT RHOBTB2 but not of the three mutants. These data indicate that the degradation of the three RHOBTB2 mutants was impaired by the CUL3 complex, but not through impaired binding with CUL3. Together all data indicating that increased amount of RHOBTB2 might be the possible underlining pathomechanism for RHOBTB2 related EE.

[Conclusion]

In conclusion, we have identified three de novo variants in RHOBTB2 as a rare genetic cause of EE, in which acute encephalopathy might be a relatively common feature. Impaired degradation in the proteasome may raise the level of mutant RHOBTB2 and thus precise regulation of RHOBTB2 levels is essential for normal brain function. Further study is needed to fully explore the pathomechanism of RHOBTB2 variants in EE.