Investigation of Sleep/Wake Regulatory Mechanisms Through the Sik3 Gene Identified by Forward Genetics

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論文概要

Dissertation Abstract

Title of Doctor Dissertation:

Investigation of Sleep/Wake Regulatory Mechanisms Through the *Sik3* Gene Identified by Forward Genetics

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Abstract

The mechanism for homeostatic sleep/wake regulation and the neural substrate for "sleepiness" remain among the biggest mysteries in sleep biology. To make a breakthrough in this issue, our laboratory has initiated a large-scale forward genetic screen of sleep/wake abnormalities in mice based on somnographic (electroencephalography (EEG)/electromyography (EMG)) measurements, which are the gold standard in mammalian sleep/wake assessment. We have so far screened > 8,000 heterozygous ENU-mutagenized mice and established multiple pedigrees exhibiting heritable and specific sleep/wake abnormalities. By combining linkage analysis and whole-exome sequencing, we have identified two dominant mutations that strongly affect sleep/wakefulness (Funato H, Honda T, Yanagisawa M et al., *Nature* 2016). A splicing mutation in the *Sik3* protein kinase gene (termed *Sleepy* mutation) causes marked hypersomnia (i.e., increased non-REM sleep (NREMS)) due to an increase in inherent sleep need. A missense mutation in the sodium leak channel NALCN (termed *Dreamless* mutation) reduces both the total amount and episode duration of REM sleep, apparently by increasing the excitability of REM sleep-inhibiting neurons. Since these dominant mutations cause severe and specific sleep abnormalities, we expect that the mutated genes play central roles in regulating sleep/wake amounts.

To elucidate the molecular basis of how the *Sik3* gene is involved in sleep/wake regulation, we performed genetic and biochemical analyses. SIK3 has a serine-threonine kinase domain at the N terminus and a protein kinase A (PKA) recognition site (Ser551) in the middle portion. The skipping of exon 13 resulted in an in-frame deletion of 52 amino acids (*ΔEx13*, termed *Sleepy* mutation) encompassing Ser551, a PKA recognition site. Thus, we hypothesized that Ser551 of *Sik3* has an essential role for the regulation of daily sleep amount and sleep need. To examine this hypothesis, we generated the transgenic mice with Ser551Ala substitution (termed *S551A* mutant)

and Ser551Asp substitution (termed *S551D* mutant) by CRISPR/Cas9 system.

As a result, *S551A* and *S551D* mutant mice both exhibited longer NREMS and decreased wakefulness, similar to the phenotype of the exon skipped *Sleepy* (*ΔEx13*) mutant. Moreover, both *S551A* and *S551D* mutant mice exhibited increased density of slow-wave activity during NREMS and increased individual NREMS episode durations, suggesting that the baseline sleep need is inherently increased in the mutants. To elucidate the mechanism of their sleep/wake phenotypes with prolonged NREMS, we performed biochemical analyses by using a series of expression plasmids (WT, *Sleepy* (*ΔEx13*), *S551A*, *S551D*) to examine how the mutations at Ser551 affect the signaling pathway and binding partners of SIK3. Through immunoprecipitation and western blotting, we confirmed that in *Sleepy* (*ΔEx13*), *S551A*, and *S551D*, all the mutants showed decreased binding to phospho-PKA substrate antibody. This result strongly suggests that the SIK3 protein with the mutations *S551A*, *S551D* and *Sleepy* (*ΔEx13*) is excluded from the possible substrates of PKA. Together, these three mutants showed abolished bindings toward 14-3-3, which corresponded to their consistent hypersomnia phenotypes with prolonged NREMS.

These results suggest that the existence of Ser551 in the *Sik3* gene and its signaling pathway, including PKA and 14-3-3, may play a key role in sleep/wake regulation under normal physiological conditions. Notably, the exon 13-encoded region of *Sik3*, including Ser551, is highly conserved among both vertebrates and invertebrates, which suggests the biological importance of the phosphorylation pathway PKA / SIK3 / 14-3-3 in sleep/wake regulation. These findings provide landmark information about the novel sleep/wake regulatory mechanisms by connecting the intracellular signaling pathway to the dynamic *in vivo* sleep/wake behaviors.