主論文の要旨

Prickle2 and Igsf9b coordinately regulate the cytoarchitecture of the axon initial segment

（Prickle2 と Igsf9b は軸索起始部の細胞構築を協調的に制御する）

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【Introduction】

Autism spectrum disorder (ASD) and epilepsy frequently coexist. Both diseases share comorbid symptoms, such as altered electroencephalogram and imbalance of the ratio of excitatory and inhibitory activity. This clinical and physiological overlap leads us to speculate a common dysfunction in the modulation of neural network.

Prickle2 has been identified in genetic studies of ASD subjects with epilepsy. A chromosomal microdeletion encompassing Prickle2 is associated with ASD and epilepsy. A whole-genome exome study found genetic variations of *Prickle2* in patients with these illnesses. These findings suggest that Prickle2 hypofunction is involved in the shared pathology of ASD and epilepsy. However, the pathological mechanism of Prickle2 remains uncertain.

The present study was conducted to investigate the pathological mechanism of Prickle2 in neurological diseases. Knockdown studies demonstrated that Prickle2 and its partner, Igsf9b, are involved in positioning of the axon initial segment (AIS), where many ASD and epilepsy susceptibility proteins accumulate.

【Materials and Methods 】

DNA constructs

Full-length mouse Prickle2 cDNA (PK2-Full, amino acids (aa) 1-886) was amplified from a mouse cDNA library and subcloned into pEGFP-C1. To visualize cell morphology, the pBact-mRFP vector was employed. In the knockdown experiments, we used short hairpin RNA (shRNA) vectors for cell biological studies. The following sequences were used to downregulate Prickle2: 5'-ATGGACAGAATAAATGGAC-3' for scramble, 5'-GCTGGAGAGAAGTTGCGAA-3' for Prickle2, and 5'-GGACCCTACTTCACGGAGT-3' for Prickle2#2. For the rescue experiment in Prickle2-knockdown neurons, we constructed an RNAi-resistant Prickle2 mutant (PK2rs) containing two mutations in the target sequence of the sh-PK2 RNAi vector.

Immunofluorescence study

Hippocampal neurons were fixed with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) for 15 min and permeabilized with PBS containing 0.2% Triton X-100 for 10 min. For the immunofluorescence study of AnkG, we permeabilized the neurons with 0.2% Triton X-100 for 1 h, followed by blocking with normal serum. The fixed neurons were incubated overnight at 4 °C with primary antibodies, washed, and incubated for 1 h with secondary antibodies. Fluorescent images were taken with a laser-scanning confocal microscope.

【Results】

The transfection of an RNAi vector encoding a short hairpin RNA targeting Prickle2 (sh-PK2) significantly suppressed expression of the *Prickle2* transgene and endogenous Prickle2

(Fig. 1a). The localization of Igsf9b was significantly different from Prickle2-knockdown neurons and control neurons transfected with sh-scramble (Fig. 1b, c). The impairment of Igsf9b localization in Prickle2-knockdown neurons was restored by the expression of RNAi-resistant Prickle2 mutant PK2rs (Fig. 1b and c). These results suggest that Prickle2 regulates the transport of Igsf9b to the neurites of hippocampal neurons.

Prickle2 knockdown also caused AnkG to redistribute in different patterns (Fig. 2a and b). Almost all the control neurons (more than 90%) showed AnkG accumulation at the proximal part of axons (Fig. 2a and b; type 1 phenotype). In contrast, AnkG was absent from the proximal part of axons in approximately 60% of prickle2-knockdown neurons (Fig. 2a and b; type 2 phenotype). The minority (approximately 30%) of Prickle2-knockdown neurons showed a discontinuous and punctate distribution of AnkG along axonal processes (Fig. 2a and b; type 3 phenotype). The expression of PK2rs restored the defective localization of AnkG (seen in phenotypes 2 and 3) in the Prickle2-knockdown neurons (Fig. 2b).

【Discussion】

In this study, we found that Prickle2 knockdown impaired the localization of AnkG and voltage-gated sodium channels, thereby affecting neural network activity. In wild-type neurons, the AIS scaffolding protein AnkyrinG (AnkG) and voltage-gated sodium channel alpha subunit (NaV) accumulate at the proximal part of axons to tune action potential. Prickle2 knockdown (PK2-KD) caused Igsf9b localization defect, resulting in the delocalization of AnkG and NaV (Fig.3). We attribute the dissociation of AIS cluster caused by the gene silencing against *Prickle2* and *Igsf9b* to the lack of intra-axonal boundary. AIS dysregulation triggered by Prickle2 hypofunction affects the control of neuronal excitability. Thus, the molecular mechanism by which Prickle2 underlies AIS dysregulation provides a positive clue for understanding shared pathogenic signaling between ASD and epilepsy.