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Mechanism for Functional Expression of the Distal Renal Tubular K^+ Channels

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Summary

【Back ground and objectives】Recent genetic analysis has revealed that the Na^+ reabsorption in the distal convoluted tubules (DCT) participates in the systemic ion homeostasis. Composing the K^+ recycling pathway for Na^+ reabsorption in DCT, distal tubular K^+ channels are principal components for the ion homeostasis. Hypertension, which reflects a state of Na^+ overload, is one of the conditions caused by derangement in the ion homeostasis. Because hypertension is one of the chief burdens of the healthcare system, clarification of the regulatory mechanism for the distal tubular K^+ channels is expected to contribute to the healthcare system.

In the previous studies, we identified the components of the distal tubular K^+ channels; members of the Membrane Associated Guanylate Kinase (MAGUK) family, Membrane Associate Guanylate kinase with Inverted domain structure 1 (MAGI-1), function as scaffolding proteins for the K^+ channels. In this study, we aimed to identify the regulatory mechanism for MAGI-1 to scaffold the K^+ channels, because the scaffolding is considered to be indispensable for the functional expression of the K^+ recycling pathway in DCT.

【Methods and Results】 *In vitro* analysis using HEK293T cells indicated that the basolateral K^+ channels/MAGI-1 interaction was disturbed by phosphorylation of the serine residue of the PDZ-binding motif of Kir4.1, a subunit of the basolateral K^+ channels. *In vivo* analysis using the anti-MAGI-1 specific antibody revealed the intra-renal basolateral K^+ channels/MAGI-1 interaction was increased under a sodium-loading condition, which is expected to reduce plasma mineralocorticoid concentration. Microarray analysis between the rats with and without continuous injection of mineralocorticoid revealed that mineralocorticoid did not change the amount of the expression in the kinases usually expressed in the kidney. *In vitro* analysis expressing Kir4.1 mutants in MDCK cells, a cell-line derived from renal distal tubules, revealed disease-causing mechanisms for the EAST syndrome. R65P, A164I and R297C, the mutants reported to be inactive at physiological pH, could be expressed on the basolateral side, whereas G77R, C140R, A167V and R199X showed diffuse intracellular distribution. In A167V, disturbed transport of Kir4.1 to MAGI-1 caused diffuse intracellular distribution. Immunohistochemical analysis using antibodies that specifically recognize isoforms of MAGI-1 revealed that a variant of MAGI-1 (MAGI-1a-short) would scaffold the apical K^+ channels in DCT.

【Conclusions】MAGI-1 isoforms participated in the functional expression of the K^+ channels in DCT: the amino-terminal longer one for the basolateral K^+ channels and the amino-terminal shorter one for the apical K^+ channels. Disruption in the process of the K^+ channels/MAGI-1 interaction, which was regulated by the phosphorylation of the interacting domains of the K^+ channels, caused tubulopathy in one lineage of the EAST syndrome. Mineralocorticoid possibly regulates the interaction by regulating the activity of the kinases expressed in DCT, such as SGK1 and WNK4. Regulation of these kinases could be a new target for hypertension treatment.