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Clarification of Molecular Mechanisms Underlying Abnormal Localization and Novel Physiological Function of Paracellular Magnesium Channel

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Summary

Magnesium is an essential cofactor for over 300 enzymes involved in metabolism and energy production. Magnesium filtrated in the glomeruli is reabsorbed through the paracellular pathway in the thick ascending limb (TAL) of Henle's loop. Claudin-16 belongs to the claudin family of tight junctional proteins and plays a critical role in the reabsorption of magnesium in the TAL. So far, we reported that the phosphoserine level of claudin-16 in hypertensive rats is lower than that in normotensive rats and urinary magnesium excretion increases in hypertensive rats. Dephosphorylated claudin-16 is mainly distributed in the cytosol, but the regulatory mechanism has not been clarified. In the present study, we searched for binding protein that regulates the distribution of claudin-16 and found that E3 ubiquitin ligase PDZ domain-containing RING finger protein 3 (PDZRN3) could bind to claudin-16.

PDZRN3 is a member of the ligand of Numb Protein-X family of RING-type ubiquitin E3 ligase. PDZRN3 plays a role in the differentiation of myoblast and osteoblast, and development of neuronal and endothelial cells. However, the role has not been examined in the kidney. In the pull-down assay, PDZRN3 was associated with wild-type claudin-16, but not with □PDZ mutant. PDZRN3 was distributed both in the cytosol and tight junctions (TJs), whereas claudin-16 was mainly distributed in the TJs in canine renal MDCK cells. H-89, a protein kinase A inhibitor, increased the association of PDZRN3 with claudin-16. Furthermore, the cytosolic PDZRN3 was co-localized with claudin-16 in the cells treated with H-89. PDZRN3 siRNA blocked the trafficking of claudin-16 from the TJs to cytosol. H-89 decreased paracellular permeability to magnesium, which was inhibited by PDZRN3 siRNA.

In conclusion, we found that PDZRN3 binds to claudin-16 and increases its cytosolic distribution. The inhibition of the function of PDZRN3 may increase the localization of claudin-16 at the TJs and magnesium reabsorption in the kidney.