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Elucidation of Mechanism of Abnormal Magnesium Reabsorption in Salt-Sensitive Hypertension

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Summary

The magnesium balance of whole body is regulated by the kidney which adapts magnesium excretion based on net magnesium absorption from intestine. Magnesium filtrated in the glomeruli is predominantly reabsorbed through the paracellular pathway in the thick ascending limb 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. 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 examined the associated protein that regulates the intracellular distribution of claudin-16 and the transcriptional regulatory mechanism of claudin-16.

Claudin-16 was endogenously expressed in OK cells derived from opossum kidney. We examined the reporter activity using the promoter of human claudin-16. The region from -2,195 to -1,682 was important to increase the reporter activity. Epidermal growth factor (EGF), a magnesiotropic hormone, increased mRNA expression by 1.5 and reporter activity by 5.3. These results suggest that EGF has little effect on the transcriptional activity of claudin-16. In the future, we are going to examine the effect of EGF on the intracellular distribution of claudin-16.

We performed a yeast two-hybrid screening for the detection of the novel associated proteins with the carboxyl region of claudin-16 and obtained several proteins such as syntaxin-8 (Stx-8) and COPS5. These proteins regulate the intracellular trafficking of several membrane proteins. In the pull-down assay, we demonstrated that the carboxyl region of claudin-16 binds to Stx-8.

In conclusion, we found that claudin-16 expression is significantly increased by EGF, but the effect is very small. The carboxyl region of claudin-16 was associated with Stx-8. In the future, we have to examine the relationship between the defect of these regulatory mechanism and pathological condition.