Abstract of Doctoral Thesis

Physiological roles of ERM protein on the kidney

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ERM (ezrin, radixin, moesin) proteins are crosslinker between membrane proteins and actin cytoskeleton. In the kidney, ezrin is highly expressed in the glomerular podocytes and apical membranes of the proximal tubules. Moesin is highly expressed in the apical membrane of the thick ascending limb of the loop of Henle (TAL), proximal tubules and endothelial cells. Here I studied the physiological roles of ezrin in the glomerular podocytes using ezrin knockdown (*Vil2*$^{kd/kd}$) mice and of moesin in TAL using moesin-null (*Msn*$^{-/y}$) mice.

Ezrin is mainly localized in glomerular podocytes and is reported to form a protein complex with podocalyxin, which is important for glomerular function. However, the physiological roles of ezrin in podocytes are still unclear. Here, I examined the importance of ezrin in the regulation of podocyte function using *Vil2*$^{kd/kd}$ mice. The *Vil2*$^{kd/kd}$ mice did not show glomerular dysfunction, morphological defects or abnormal localization of podocalyxin in podocytes. However, I found that *Vil2*$^{kd/kd}$ mice showed reduced susceptibility to glomerular injury. In *Vil2*$^{kd/kd}$ mice, Rac1 activity was significantly reduced and RhoA activity was significantly increased compared to wild type (WT) mice.

Moesin is highly expressed in TAL and is reported to interact with Na^+-K^+-$2Cl^-$ cotransporter type 2 (NKCC2), which plays important roles in regulating fluid balance in TAL. However, the physiological roles of moesin in TAL are still remain unclear. Here, I examined the physiological function of moesin in the kidney using *Msn*$^{-/y}$ mice. *Msn*$^{-/y}$ mice exhibited mild hyperchloremia, and reduced glomerular filtration rate compared to WT mice. I found that apical surface expression of NKCC2 was significantly increased in *Msn*$^{-/y}$ TAL. Subcellular fractionation of renal medulla lysate and internalization assay using tubular suspension showed that the process of NKCC2 endocytosis is impaired. Since the distribution of NKCC2 in lipid raft fractions was decreased in *Msn*$^{-/y}$ mice, moesin may regulate the NKCC2 distribution to microdomain. These results suggest that moesin regulates the

internalization of NKCC2.

In conclusion, loss of ezrin protects podocytes from injury-induced morphological changes by reducing of Rac1 activity and increasing of RhoA activity in glomeruli. In TAL, moesin regulates the apical surface expression of NKCC2 by targeting NKCC2 to lipid raft, and plays important roles in the renal electrolyte handling.