

Abstract of Doctoral Thesis

Physiological roles of the cytoskeletal protein ezrin in the gastrointestinal tract

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Ezrin is mainly expressed at the brush border membrane (BBM) of gastrointestinal tracts and renal urinary tubules, and cross-links membrane proteins with actin cytoskeleton. It is involved in morphological changes of cells accompanied with the remodeling of actin filament, and in the functional expression of membrane protein complexes at the cell surface. Here I studied the functional roles of ezrin in the gastrointestinal tracts by using transgenic ezrin knockdown (*Vil2^{kd/kd}*) mice in which expression level of ezrin was decreased to <5% compared with wild-type mice. I newly examined the physiological roles of ezrin by comparing the tissues of the wild-type and *Vil2^{kd/kd}* mice and by performing proteome analysis of their BBM fractions.

In the stomachs, ezrin is predominantly expressed on the apical canalicular membrane of parietal cells. Previously, it had been shown that the *Vil2^{kd/kd}* mice showed achlorhydria due to the impairment of membrane fusion between tubulovesicles and apical membrane. Here, I newly found that the *Vil2^{kd/kd}* mice showed hypergastrinemia and foveolar hyperplasia in the gastric fundic region. Dilation of fundic glands was observed with the percentage of parietal and chief cells being decreased, and that of neck cells being increased. The parietal cells of the *Vil2^{kd/kd}* mice contained dilated tubulovesicles and abnormal mitochondria, and subsets of these cells contained abnormal vacuoles.

In the small intestines, ezrin is co-localized with many transport proteins on the BBM of absorptive epithelial cells. It is hypothesized that ezrin is involved in stable expression of transport proteins at the cell surface. However, comprehensive analysis has not been performed yet. Here, I newly prepared the BBM fractions of ileums from the wild-type and *Vil2^{kd/kd}* mice, analyzed them by mass spectrometry, and compared their proteomic patterns. Several transport proteins, cytoskeleton-associated proteins, and trafficking proteins were up- or down-regulated in the BBM fraction of the ileum of the *Vil2^{kd/kd}* mice. Among them, down-regulation of sodium monocarboxylate transporter 1 (SMCT1), chloride intracellular channel protein 5 (CLIC5), and a scaffold proteins Na⁺/H⁺ exchanger regulatory factors 1 (NHERF1) was confirmed by western blotting and immunofluorescence.

In conclusion, ezrin is involved in not only gastric acid secretion but also in the maintenance of the structure of the parietal cell and the gastric mucosa. In the ileum, ezrin is involved in the stable expression of transport proteins at the cell surface.