Nedd4-2, ClC-5 and albumin endocytosis in the proximal tubule: a role for SGK-1?

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Retrieval of urinary albumin by the proximal tubule is achieved by receptor-mediated endocytosis that involves a macromolecular complex that includes megalin/cubulin receptor, the Cl^- channel ClC-5, Na-H exchanger isoform 3 (NHE3) and v-H^+-ATPase. Defects in this uptake pathway result in increased albumin excretion, albuminuria and eventually nephropathy. Genetic defects in ClC-5 in patients with Dents disease lead to persistent proteinuria. ClC-5 knockout mice also have proteinuria, demonstrating a key role for ClC-5 in this process. ClC-5 is expressed at the apical cell membrane of the proximal tubule and we have been investigating the molecular basis for the role of ClC-5 in albumin uptake. ClC-5 has a large intracellular C-terminus that can potentially act with numerous regulatory proteins. Previously it has been demonstrated that the cell surface expression of ClC-5 can be regulated by an ubiquitin ligase, WWP2. In the current study we showed that Nedd4-2 interacts with ClC-5 and that this interaction is an essential component of constitutive albumin uptake (Hryciw *et al.*, 2004). We also investigated whether serum- and glucocorticoid-inducible kinase (SGK-1) plays a role in this endocytic process.

We first used Glutathione S transferase (GST) fusion pulldowns to show that C-terminus of ClC-5 bound both Nedd4 and Nedd4-2. The Xenopus oocyte expression was then used to show that Nedd4-2 but not Nedd4 reduced ClC-5 currents in a manner that was dependent on an intact proline rich motif containing a tyrosine (PY) in ClC-5. Using luminescence detection of an influenza hemagglutinin-HA-epitope-tagged ClC-5, the decrease in ClC-5 currents was confirmed to be due to a reduction in cell surface levels of ClC-5. Acute exposure of opossum kidney (OK) cells to albumin resulted in a rapid increase in the protein levels of both ClC5 and Nedd4-2 and an increase in proteasome activity. Conversely, inhibition of the proteasome or silencing of endogenous Nedd4-2 in OK cells caused significant decreases in albumin endocytosis. These data indicate that constitutive albumin uptake involves the upregulation of the ubiquitin/proteasome system

In the cortical collecting duct, it is hypothesized that SGK inhibits the action of Nedd4-2 on ENaC. We therefore investigated the role of SGK-1 on albumin uptake in OK cells. Overexpression of wildtype SGK-1 resulted in a significant increase albumin endocytosis 114 ± 3.5%; n = 4; P Our data clearly demonstrate that, similar to the reabsorption of Na^+ by the cortical collecting duct, that the constitutive uptake of albumin involves ubiquitin ligases, the proteasome and SGK-1. However, it appears that the effects of SGK-1 do not involve either Nedd4-2 or ClC-5 in OK cells. These data highlight the complex nature of the endocytic process that mediates the retrieval of albumin from the glomerular filtrate.

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