신 집합관 수분통로 AQP2 조절 E3 ubiquitin-protein ligases 발굴

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E3 Ubiquitin-protein Ligases Associated with AQP2 Regulation in Kidney Collecting Duct

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Ubiquitination is important for endocytosis and lysosomal degradation of AQP2. We aimed to identify E3 Ubiquitin-protein ligases which could be associated with AQP2 regulation in the kidney. Sprague-Dawley rats were infused with vehicle (n=13), dDAVP for 5 d (n=13), or dDAVP was withdrawn in rats for periods (15 min, 30 min, 1 h, 3 h, 6 h, 12 h, or 24 h) after 5 d-dDAVP infusion (n=46) for inducing AQP2 endocytosis and degradation. Immunoisolated AQP2- or Ub-expressing plasma membrane (PM)- and intracellular vesicle (ICV)-enriched membrane fractions of whole kidney were trypsin-digested and subjected to LC-MS/MS analysis. Protein samples were quantified by a label-free method using MS<E> mode. dDAVP treatment for 5 d (D5d) significantly decreased urine output and increased urine osmolality. Increased urine osmolality was maintained during 3 h-withdrawal of dDAVP (D5d-3h), and thereafter it was decreased to the control levels. Consistent with this, increased AQP2 levels in the PM-fractions were only seen in the D5d and D5d-3h, whereas AQP2 levels in the ICV-fractions began to increase after 3 h-dDAVP withdrawal. LC-MS/MS analysis identified seven isoforms of E3 ligases (UBR4, UBR5, UHRF1, NEDD 4, MIB2, BRE1B, Cullin-5) in AQP2- expressing PM- and ICV-fractions, and RT-PCR demonstrated mRNA expression of these E3 ligases in whole kidney and inner medulla. Time-course of the changes in the expression was examined, based on their peak intensities of digested peptides and the number of peptides. Moreover, expression levels of BRE1B and Cullin-5 were examined by immunoblotting and immunohistochemistry. Taken together, dDAVP-induced AQP2 was significantly internalized during dDAVP withdrawal and this might be degraded through an activated ubiquitin-proteasome pathway, possibly mediated by identified E3 ligases. Further studies including siRNA-mediated gene silencing of identified E3 ligases in collecting duct cells are needed to examine their specificity to AQP2 degradation.

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