KSN 2016 Abstract Submission

Volume, Acid-Base & Electrolyte

KSN2016ABS-1248

Vacuolar protein sorting-associated protein 35 is associated with lysosomal degradation of aquaporin-2 in kidney collecting duct cells

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Background: Carboxyl-terminus of aquaporin-2 (AQP2c) undergoes post-translational modifications, including phosphorylation and ubiquitination, for the regulation of aquaporin-2 (AQP2) translocation and protein expression in the kidney collecting duct cells. We aimed to identify novel proteins interacting with AQP2c.
Methods: Recombinant rat AQP2c protein was made in *E. coli* BL21 (DE3) by exploiting the pET32 TrxA fusion system. Lysates of rat kidney inner medullary collecting duct (IMCD) tubule suspensions were interacted with rat AQP2c bound to Ni^{2+}-resin. LC-MS/MS proteomic analysis demonstrated 18 proteins, including vacuolar protein sorting-associated protein 35 (Vps35).
Results: Co-immunoprecipitation assay demonstrated that Vps35 interacted with AQP2c. Immunohistochemistry revealed that AQP2 and Vps35 were co-localized at the intracellular vesicles in the rat kidney collecting duct cells. The changes of intracellular translocation and protein expression of Vps35 in the kidney, however, were not seen in response to water restriction in rats, where the changes of AQP2 expression were evident. The role of Vps35 in the dDAVP-induced AQP2 regulation was examined in mpkCCDc14 cells. Cell surface biotinylation assay demonstrated that dDAVP-induced apical translocation of AQP2 was significantly decreased under the siRNA-mediated Vps35 knockdown. dDAVP-induced AQP2 up-regulation was seen to a lesser extent in the cells with Vps35 knockdown, when compared with control. Moreover, AQP2 expression was decreased to a greater extent during the withdrawal period after dDAVP stimulation under the Vps35 knockdown, which was significantly inhibited by chloroquine (a blocker of the lysosomal pathway) treatment, but not by MG132 (a proteasome inhibitor) treatment.
Conclusion: Taken together, Vps35 is an interacting protein with AQP2c and depletion of Vps35 is likely to increase the lysosomal degradation of AQP2 protein.

Keywords: aquaporin-2, collecting duct , Lysosome, Retromer, Vps35