Abstract Type : Oral
Abstract Submission No. : 1392

Poly(ADP-ribose) polymerase 1 affects the vasopressin-mediated AQP2 expression via an interaction with β-catenin

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Objectives: Poly(ADP-ribosy)lation (PARylation) mediated by poly(ADP-ribose) polymerases (PARPs), which catalyzes the transfer of ADP-ribose from NAD^+ molecules to acceptor proteins, regulates diverse cellular processes. Since PARP1 gene-deficient mice revealed an increase in urine volume, we aimed to examine the role of PARP1, the most abundant protein in the PARPs family, in the AQP2 regulation.

Methods: 1) Immunoblotting for PARP1 in mpkCCDc14 cells; 2) Pulldown assay of biotin-conjugated NAD^+ and immunoprecipitation (IP) assay using poly(ADP-ribose) (PAR) antibody; 3) qRT-PCR and immunoblotting for AQP2; and 4) Bioinformatics for elucidating PARP1-interacting proteins in kidney collecting duct (CD) cells.

Results: Immunoblots showed that dDAVP treatment (10^{-9} M) induced the cleavage of PARP1 (89 kDa and 25 kDa) in mpkCCDc14 cells. dDAVP treatment (10^{-9} M, 24 h) also increased the abundance of total PARylated proteins in biotin-NAD^+ pulldown and IP assays of PAR in mpkCCDc14 cells. On the other hand, siRNA-mediated PARP1 knockdown significantly attenuated the dDAVP-induced AQP2 mRNA and protein abundance, suggesting a role of PARP1 in AQP2 regulation. In contrast, the PARP1 inhibitor (PJ34) did not reduce the dDAVP-induced AQP2 abundance, despite the significant decrease in the PARylation. The results suggest that dDAVP-regulated AQP2 expression is associated with PARP1 protein *per se*, but not with PARP1-mediated PARylation. Bioinformatics study revealed that 408 proteins interact with PARP1 in the kidney CD cells. Among them, 49 proteins were mapped on the vasopressin V2 receptor (V2R) signaling pathway. In particular, β-catenin, which is phosphorylated (S552) by dDAVP, was identified as the PARP1-interacting protein mapped on the V2R signaling. Immunoblotting demonstrated that siRNA-mediated knockdown of PARP1 was associated with decreased dDAVP-induced phosphorylation of β-catenin at S552 in mpkCCDc14 cells.

Conclusions: PARP1 is likely to play a role in vasopressin-mediated AQP2 regulation via the protein interaction with β-catenin rather than PARylation of proteins in the kidney CD cells.