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TAZ-knockdown affects the vasopressin-induced aquaporin-2 (AQP2) trafficking and protein abundance in kidney collecting duct cells

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Objectives: Transcriptional coactivator with PDZ-binding motif (TAZ), a downstream effector of the hippo signaling pathway, regulates the expression of target genes by acting as a transcription cofactor. TAZ KO mice were known to display multicystic kidneys with polyuria. We aimed to study the role of TAZ in vasopressin-induced AQP2 regulation.

Methods: 1) siRNA-mediated knockdown of TAZ in mpkCCDc11 cells; 2) RT-qPCR, semiquantitative immunoblotting, immunocytochemistry of AQP2; 3) Next Generation Sequencing (NGS) in mpkCCDc11 cells, the mouse collecting duct cell line.

Results: Endogenous AQP2 expression was induced in mpkCCDc11 cells by dDAVP (10^{-9}M) treatment. After starvation for 24 h, dDAVP (10^{-9}M) stimulation for 15 and 30 min induced a significant AQP2 translocation to the cell membrane. In contrast, the dDAVP-induced AQP2 translocation was markedly attenuated in the cells with siRNA-mediated TAZ knockdown (TAZ-KD), despite no changes in cAMP production. Phalloidin staining demonstrated the excessive stress fiber formation in the TAZ-KD. dDAVP (10^{-9} M) treatment for 24 h induced AQP2 mRNA (12,608± 177% of the control) and AQP2 protein abundance (270 ± 18%). In contrast, dDAVP-induced increase of AQP2 mRNA (273 ± 14% of the control) and protein abundance (99 ± 17%) was significantly attenuated in TAZ-KD. Unchanged TonEBP protein abundance was observed in TAZ-KD. NGS identified several potential AQP2 transcription factors (TF), and Klf6, Irf3, Cebpb, and Nr4a1 were selected based on previous in silico database. Among them, Nr4a1 was chosen for further studies due to a significant decrease in the mRNA expression levels in TAZ-KD, as demonstrated by RT-qPCR.

Conclusions: TAZ is likely to affect dDAVP-induced AQP2 trafficking. This is not mediated by the changes in cAMP/PKA pathway, but other non-canonical pathways are involved. TAZ could regulate AQP2 abundance, possibly via an interaction with several TF.