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Single-tubule RNA-Seq uncovers signaling mechanisms that defend against hyponatremia in syndrome of inappropriate antidiuresis

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The syndrome of inappropriate antidiuresis (SIAD) is the most frequent cause of dilutional hyponatremia in hospitalized patients and leads to high morbidity and mortality. In SIAD, the extent of hyponatremia is limited by onset of vasopressin escape caused by loss of the water channel aquaporin-2 (Aqp2) in the renal collecting duct despite high level of circulating vasopressin. The transcriptional regulatory mechanisms and signaling pathways responsible or vasopressin have remained unknown for decades, although prior works documented evidence that the protein expression of Aqp2 decreases in response to an extra water load. The chief limiting factor in the investigation of vasopressin escape is the fact that collecting duct principal cells only account for a small fraction of the renal cortex, making standard biochemical and systems-biology techniques largely unfeasible. Here, we combine single-tubule RNA sequencing (RNA-seq) with the methods of systems biology in a well-established rat model of SIAD to identify signaling pathways activated at the onset of vasopressin escape. Using single-tubule RNA-seq, full transcriptomes were determined in microdissected cortical collecting ducts of vasopressin-treated rats at 1, 2, and 4 days after initiation of oral water loading in comparison to time-control rats without water loading. The time-dependent mRNA abundance changes were mapped to gene sets associated with curated canonical signaling pathways and revealed evidence of perturbation of transforming growth factor β signaling and epithelial-to-mesenchymal transition on Day 1 of water loading simultaneous with the initial fall in Aqp2 gene expression. On Day 2 of water loading, transcriptomic changes mapped to Notch signaling and the transition from G0 into the cell cycle but arrest at the G2/M stage. There was no evidence of cell proliferation or altered principal or intercalated cell numbers. Exposure of vasopressin-treated cultured mpkCCD cells to transforming growth factor β resulted in a virtually complete loss of Aqp2. Thus, there is a partial epithelial-to-mesenchymal transition during vasopressin escape with a subsequent shift from quiescence into the cell cycle with eventual arrest and loss of Aqp2. Our observations are consistent with a prior report that suggests high plasticity of renal cortical collecting ducts regarding phenotype transition between epithelial and mesenchymal cells.