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RNA-seq in microdissected collecting ducts reveals increased expression of cell-cycle genes in early stages of vasopressin escape

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Background: Vasopressin escape is an important protective mechanism against hyponatremia. Although downregulation of aquaporin-2 gene expression in the whole kidney has been well documented, coregulated transcripts in vasopressin escape have not been identified. Furthermore, transcriptional changes at the level of single tubule have never been explored before, mainly due to the lack of an unbiased and highly sensitive profiling technique. Recent advances in next-generation sequencing technologies have enabled researchers to investigate transcriptomic changes occurring in single cells and a small number of cells obtained from biopsies or microdissection.

Methods: Combining RNA-seq and classical microdissection, we investigated transcriptomic changes occurring in the CCD of rats undergoing vasopressin escape. Male Sprague Dawley rats receiving a sustained dose of dDAVP were given either high water load (50 mL/day) or a lower water load (25 mL/day) matching insensible losses plus a small urinary output. Rats were humanely euthanized at days 1, 2, and 4 (post-water load). CCDs were collected by manual microdissection and transcriptionally profiled using RNA-seq.

Results: Downregulation of aquaporin-2 and aquaporin-3 transcripts took place on day 1 of water loading and persisted through day 4, consistent with time courses of urine osmolality and water excretion changes in the same rats. Differential expression analysis of the global transcriptomic changes revealed increases in many transcripts related to cell cycle and DNA replication (selectively confirmed by RT-PCR). When quiescent cultured mpkCCD cells were switched from Go to the cell cycle by serum addition, aquaporin-2 protein abundance was markedly diminished. Consistent with the findings in CCD RNA-seq and cultured mpkCCD cells, immunofluorescence labeling of microdissected CCDs for V-ATPase and pendrin revealed an increase in the proportion of cells undergoing division, and no significant changes in the proportions of principal and intercalated cells. These findings suggest that vasopressin escape involves cell division in the CCD, although it is uncertain whether this phenomenon actually leads to remodeling of the CCD.

Conclusion: Along with downregulation of aquaporin-2 and aquaporin-3, a substantial number of mRNA transcripts changed significantly in the early time course of vasopressin escape. Many of these transcripts are

related to cell division and DNA replication. Increased cell division in the CCD and suppression of aquaporin-2 expression in cells entering cell cycle point to a dynamic process involving a change in the proportion of aquaporin-2-expressing cells in the CCD.

Keywords: cortical collecting duct, RNA-seq, transcriptome, vasopressin escape