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Perspective

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Are Some Nephrons More Equal Than Others?: Perspective on "Viewing Cortical Collecting Duct Function Through Phenotype-Guided Single-Tubule Proteomics"

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The human kidney consists of about a million nephrons, arranged in parallel. Although each nephron contains multiple segments that do different things, one generally assumes that each parallel unit has qualitatively and quantitatively the same functions carried out by the same proteins (except for the obvious distinction between cortical and juxtamedullary nephrons). This, however, is a default assumption, supported mainly by the lack of detailed information on differences among all these anatomically similar structures. An article by Himmerkus et al.,[1] in this inaugural issue of *Function*, starts from a contrary position that defined tubular segments may have important quantitative differences in both function and protein expression.

The group previously published a study[2] using state-of-the-art mass spectroscopy to assess proteins in individual glomeruli and proximal tubules from mouse and human kidneys. The approach identified more than 1500 different proteins in an isolated (proximal) tubule. Although not yet as sensitive as techniques that read RNA transcripts (Figure 1), expression of protein should reflect cell function more closely than that of mRNA. This feat depended on major advances in both sample preparation and instrumentation.

This article carries this one step further by examining both tubular function (appropriately enough for this journal) and protein expression in the same isolated segment. For this, they chose the cortical collecting duct. This part of the renal tubule contains three or more different cell types and regulates levels of cations (Na^+, K^+, H^+), anions (Cl^-, HCO_3^-), and H_2O by varying rates of transport between urine and blood[5,6] (Figure 1). It is an ideal place to start looking for correlates between function and protein expression.

For starters, they measured amiloride-sensitive voltage (reflecting ENaC-mediated Na^+ reabsorption), transtubular resistance and diffusion potentials (reflecting tight-junction permeability and its selectivity) in isolated, perfused CCDs. Not unexpectedly for such physiological experiments, ΔV_{amil} ranged 3-fold, from −7 to −30 mV. In the same tubules, they assessed a panel of 17 proteins, including very abundant ones such as the

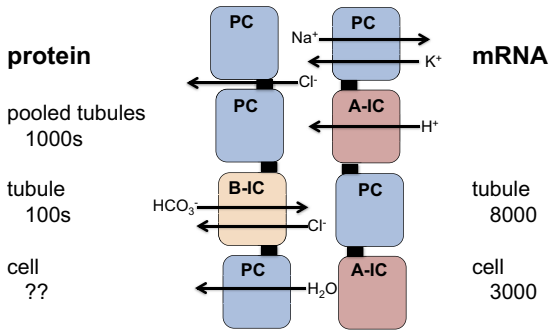

Figure 1. Functions and Omics of the Cortical Collecting Duct. This tubular segment helps control the volume and composition of body fluids by regulating transport of ions and water. Numbers for mRNA and protein species are detected from Chen et al., Himmerkus et al., Höhne et al., and Lee et al.[1–4] PC, principal cell; A-IC, A-type intercalated cell; B-IC, B-type intercalated cell.

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Na/K-ATPase and relatively scarce ones such as the β-subunit of ENaC. These also showed considerable variability. Having both datasets from the same tubules permitted calculations of correlations between different parameters. For example, expression of the beta subunit of the epithelial Na channel (βENaC) correlated positively with ΔV_{amil}, which at first seems logical but is nonetheless interesting since the βENaC is not much altered by aldosterone, the main regulator of ENaC in the kidney.[7]

Other relationships were more surprising, particularly the negative correlation between ΔV_{amil} and the expression of aquaporin 2 (AQP2), the water channel that inserts into the apical membrane of principal cells to control ADH-dependent H_2O transport and urine osmolarity.[5] This suggests that some CCDs may commit to Na transport or to H_2O transport at the expense of the other, although a lot more work will need to be done to sort this out.

Another strength of the approach entails the definition of sets of proteins that change their expression together. B-type intercalated cells use the anion exchanger pendrin to secrete HCO_3^- into the urine in exchange for Cl^-, particularly during alkalosis.[8] Expression of pendrin correlated positively with that of the B2 subunit of the vacuolar ATPase that pumps H^+ from the cell to the blood and with barttin, a regulator of the anion channels that move Cl^- from cell to blood to maintain electroneutrality. All of these proteins are part of the same overall transport pathway, and also tend to change in concert when the pendrin gene is knocked out. These responses to genomic manipulation may give clues about causalities underlying the correlations. More surprising was the positive correlation with another anion exchanger, slc4A1 (AE1), which resides on a different cell type, namely the A-type intercalated cell, and mediates urinary acidification. This and other findings argue against the simple idea that the correlations simply reflect changes in the numbers of various cell types, a phenomenon known to occur in the CCD.[9]

These results demonstrate the possibility of studying variations in protein expression and function among tubular segments from the same animal under nominally identical conditions. This would both define differences in physiology among nominally identical structures and help to explain those differences in terms of protein abundance. Ultimately, this approach could be applied at the level of the single renal cell, as is currently done with transcriptomics. With advances in the sensitivity of proteomics techniques, this achievement may not be far away.

Conflict of interest statement

The author has no conflicts of interest to declare.

References

1. Himmerkus N, Svendsen S, Quitanova C et al. Viewing cortical collecting duct function through phenotype-guided single-tubule proteomics. *Function* 2020;1. doi:10.1093/function/zqaa007.
2. Höhne M, Frese CK, Grahammer F et al. Single-nephron proteomes connect morphology and function in proteinuric kidney disease. *Kidney Int* 2018;93(6):1308–1319.
3. Chen L, Lee JW, Chou CL et al. Transcriptomes of major renal collecting duct cell types in mouse identified by single-cell RNA-seq. *Proc Natl Acad Sci USA* 2017;114(46):E9989–E9998.
4. Lee JW, Chou CL, Knepper MA. Deep sequencing in microdissected renal tubules identifies nephron segment-specific transcriptomes. *J Am Soc Nephrol* 2015;26(11):2669–2677.
5. Pearce D, Soundararajan R, Trimpert C, Kashlan OB, Deen PM, Kohan DE. Collecting duct principal cell transport processes and their regulation. *Clin J Am Soc Nephrol* 2015;10(1):135–146.
6. Roy A, Al-bataineh MM, Pastor-Soler NM. Collecting duct intercalated cell function and regulation. *Clin J Am Soc Nephrol* 2015;10(2):305–324.
7. Ergonul Z, Frindt G, Palmer LG. Regulation of maturation and processing of ENaC subunits in the rat kidney. *Am J Physiol Renal Physiol* 2006;291(3):F683–F693.
8. Wall SM, Verlander JW, Romero CA. The renal physiology of pendrin-positive intercalated cells. *Physiol Rev* 2020;100(3):1119–1147.
9. Assmus AM, Mullins JJ, Brown CM, Mullins LJ. Cellular plasticity: a mechanism for homeostasis in the kidney. *Acta Physiol (Oxf)* 2020;229(1):e13447. doi is 10.1111/apha.13447.