REPLY TO EDEMIR:

Physiological regulation and single-cell RNA sequencing


Tacitly, Edemir (1) points to an important issue in the interpretation of data from single-cell RNA-sequencing experiments. Any given cell can exist in a variety of regulatory states that are affected both by extracellular signals and the prior history of the cell. Consequently, data from single-cell RNA sequencing, such as carried out in our paper (2), provide only a partial characterization of a given cell type. Workers engaged in large-scale attempts to identify transcriptomes of every cell type in the body (3) would do well to take note of this constraint. However, the validity of such Cell Atlas projects is supported by the observation that physiological regulation in metazoan cells is generally achieved through incremental changes in the regulatory state of numerous genes and their proteins and not by selective induction or ablation of expression of one gene or another. Thus, we would expect that the genes expressed in a given cell type will be relatively unaffected by physiological factors, although relative mRNA levels would be expected to vary.

In considering the potential use of single-cell RNA sequencing for studies of physiological regulation as proposed by Edemir, there are important caveats. First of all, when studying epithelial cells, it must be remembered that cell–cell interactions between neighboring epithelial cells create important signals that influence transcription, e.g., ZO-1 from tight junctions (4), β-catenin from adherens junctions and desmosomes (5), integrin-linked signaling molecules (6), and Yes-associated protein (YAP) from both tight junctions and adherens junctions (7, 8). The process of isolating single cells necessarily removes these signals, potentially interfering with physiological responses under study. Beyond this, tissue dissociation and cell isolation may create adverse conditions related to loss of nutrients or altered oxygen availability, a point made recently by Potter and colleagues (9). Thus, metabolically active cell types like proximal tubule cells and cells from the thick ascending limb of the loop of Henle may be adversely affected, altering their gene expression profiles, while cells that are metabolically more stable like collecting duct cells in our study may be less vulnerable to stress. Therefore, it may be wise to conduct studies of physiological regulation in intact epithelia rather than in single cells. We have shown (10) that it is feasible to carry out transcriptomic profiling in single microdissected renal tubules based on techniques developed many years ago for rapid dissection of viable kidney tubule segments (11). This is the approach that we recommend to Edemir and others wishing to study physiological regulation in epithelial cells of the kidney.


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