Proteomics reveal the breadth and limits of model systems inferences.

Focus on “Proteomic analysis of V-ATPase-rich cells harvested from the kidney and epididymis by fluorescence-activated cell sorting”

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Physiologists of many generations have employed tractable model systems to make observations and then have extrapolated those observations to other more complex or less amenable systems. Such an approach is built on initial obvious similarities of the systems in form or function. The success of this philosophical approach has been mixed. Depending on the tissue, species, and especially depending on the function being assessed, there has been a variety in the levels of success. Thus, one can easily be suspect of such comparisons. Over the past two decades many reports have highlighted the similarities or hypothesized similarities between intercalated cells of the nephron and narrow or clear cells of the epididymis (2). The rationale for the comparison is the knowledge that both cell types are known to contain a substantial number of mitochondria, and that both cells types have a major role in secreting \( H^+ \) into their respective ducts. The nephron regulates systemic \( pH \) by secreting \( H^+ \) or base equivalents, and the epididymis generates an acid luminal environment that promotes sperm maturation and allows for sperm storage. Routine techniques have revealed substantial overlap between these forhead-related (FORE) cells, a class of cells that also includes interdental cells of the inner ear, in which Foxi1 is required for the expression of specific vacuolar (V) \( H^+ \)-ATPase subunits A1, B1, E2, and a4 (7). Each of these cell types reportedly expresses, in a regulated manner, V-type \( H^+ \)-ATPase in its apical membrane, where it is thought to be required for \( H^+ \) secretion. Other well-documented similarities between these cells include the expression of NBC3 and carbonic anhydrase 2 (4, 6). Surprisingly, however, interdental cells appear to secrete little acid (Miyazaki H, Marcus DC, and Wangemann P, personal communication). Nonetheless, based on the success of identifying similarities between these cell types thus far, a number of laboratories are continuing with lines of investigation built on the supposition that numerous additional parallels exist between these cell types in the kidney and the male reproductive duct.

The cell isolation and proteomic approach taken by Da Silva et al. (3) seems to break new ground in the correlative study arena. The tack of hypothesizing functional correlates and then searching for individual proteins or regulatory cascades is an inefficient strategy to define commonalities and differences between cells. The new approach described by the authors is philosophically simple, but technically challenging—isolate pure populations of FORE and non-FORE cells from both the kidney and the epididymis followed by proteomic analysis of those cells. Expression of enhanced green fluorescent protein driven by the ATP1B1 promoter was used to “tag” FORE cells for sorting (5). (Interdental cells in the mouse inner ear were also “tagged” by this approach, although the cells were not analyzed in the current report.) A simple \( 2 \times 2 \) factorial design allows for comparisons of proteins identified in FORE and non-FORE cells within a tissue and for comparisons between FORE cells across tissues. The tacit assumption is that this proteomic approach will reveal areas of broad similarity and of mechanistic identity between these cell populations.

This approach has produced a bonanza of data for which the cell biology community should be thankful. Perhaps the most important outcome is the identification of 1,564 and 2,297 proteins expressed in epididymal and renal FORE cells, respectively (full lists are available at the National Heart, Lung, and Blood Institute Laboratory of Kidney and Electrolyte Metabolism website). The authors provide an excellent initial analysis of the components, functions, and processes represented by the proteins that were identified. As expected, this analysis reveals substantial overlap in the characteristics of proteins identified in these two cell types. However, a closer inspection and parsing of the data reveal quite illuminating insights (Fig. 1). First, 875 of the identified proteins are present in FORE cells in both tissues, which accounts for roughly one half to one third of the proteins identified. Thus, the complement of proteins that makes each of these cell types unique, one half to two thirds, is quite substantial. Clearly, a sizable repository of data is available to be mined or dissected to reveal unique capabilities and unique regulation of the FORE cells derived from each tissue. A second goal addressed by the authors was to determine the identity of proteins that were present in greater abundance.

![Fig. 1. Distribution of FORE cell proteins. Upper area represents proteins present in greater abundance.](http://www.ajpcell.org)
abundance in FORE cells of each tissue. This population accounted for approximately 10% of all identified proteins. One might readily speculate that the proteins present in greater proportion are key to giving functional identity to this class of cells. Thus, once again, the authors have provided a resource to the community in the form of a list of high-priority targets. The role, contribution, and requirement for each of these proteins must be established since they appear to be key in establishing the essence of FORE cells.

Perhaps the most striking conclusions that can be drawn from the Da Silva et al. report are the stark differences between intercalated cells and narrow/clear cells when individual proteins are assessed. Less than 2% of all proteins identified, a total of 33 proteins, are common to both cell types and are in greater abundance than in non-FORE cells. The list provided by the authors includes three V-type H\(^{+}\)-ATPase subunits and seventeen mitochondrial proteins. These observations are not surprising given that the cells were selected on the basis of the expression of a V-type H\(^{+}\)-ATPase and since it is known that these cells harbor a disproportionately high number of mitochondria. The remaining list is short and diverse, ranging, for example, from a progesterone receptor to selected keratins and gelsolin and on to ubiquitin B and a predicted ubiquitin-like protein. Importantly, it has already been reported that gelsolin plays a critical role in V-type H\(^{+}\)-ATPase trafficking in intercalated cells (1), which provides evidence to support the veracity of this short list. Nonetheless, the FORE cells present in the kidney and epididymis seem to be defined by a very short list of universal proteins. The proteomic analysis shows that most proteins are expressed in a tissue-specific pattern. Such a pattern of expression will equip the cells to serve unique functions or to respond to unique stimuli in each organ.

The proteomic approach provides a wealth of new information that readily serves as an hypothesis generator. Using this approach, Da Silva and colleagues have provided additional evidence for similarities of some functions between FORE cells in the kidney and epididymis. The differences that are revealed, however, provide strong evidence that it is imperative to fully evaluate the activity of these cells in the context of their native tissue as one seeks to address male factor infertility, distal renal tubular acidosis, and other diseases that may be associated with these unique acid-secreting cells.

GRANTS

The author is supported by National Institutes of Health Grant R01-HD-058398.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author.

REFERENCES