Considerations in Comparing Proximal Tubule Cell Culture Models

The use of immortalized and primary cell cultures to study the proximal tubule (PT) has significantly enhanced our understanding of how this nephron segment functions under normal and diseased conditions. The recent publication in *JASN* of a transcriptomic comparison of 14 available PT cell models from six species[1] provides a great opportunity to have a thoughtful discussion about best practices in model selection, experimental design, and interpretation of studies involving cell lines. In their letter to the editor, Devuyst and colleagues compare primary mouse PT cultures (mPTCs) to commonly used human, porcine, and opossum cell lines (HK-2, LLC-PK1, and OK cells, respectively).[2] On the basis of their assessment of endocytic protein abundance, albumin uptake, and proliferation index, they conclude that "compared with mPTCs, immortalized cell lines displayed considerably lower levels of endosomal/lysosomal … and PT differentiation markers, contrasting with increased levels of regulatory proteins involved in proliferation and cell cycle" and suggest "a set of biologic features, addressing the interplay between proliferation and differentiation, may help identifying physiologically relevant PT cell systems." We agree completely that a more comprehensive checklist of how well individual PT cell models recapitulate cell function is warranted. However, we have concerns about the interpretation of the data provided to support the pre-eminence of mPTCs over immortalized cell lines as more differentiated and endocytically active. The authors' implication that immortalized PT cells uniformly fail to elaborate a functional apical endocytic machinery is both contrary to what is generally appreciated in the field and to the conclusions of Khundmiri *et al.* on the basis of their transcriptional profiling.[1]

The authors use immunoblotting to quantitatively compare protein levels from cell lines of different species (mouse, human, pig, opossum), using antibodies that were not validated to target conserved regions within the proteins examined with equivalent affinity. As one example, Lamp1 antibodies are notoriously species selective, and it is no surprise that anti-mouse Lamp1 antibody does not recognize Lamp1 in the other cell lines. Moreover, the expression of Rab proteins does not generally correlate with endocytic activity. For example, we find only a small difference in Rab11a protein and mRNA expression between partially and fully differentiated OK cells, despite a dramatic elaboration of apical endocytic compartments and a five-fold enhancement in endocytic uptake in the latter.[3,4] In the absence of antibodies that recognize all species identically, we have found that droplet digital PCR provides a straightforward way to quantitatively compare transcript levels of proteins in different species, and demonstrate that megalin and cubilin transcripts in OK cells are expressed at levels comparable to those in mouse kidney cortex.[5] Although mRNA data do not necessarily translate to protein levels, the ratios of megalin to cubilin mRNA and protein expression in microdissected rat nephron PT segments correlate quite well.[6,7]

A second concern is the apparent absence of albumin uptake in LLC-PK1 and OK cells, which have been used for decades to study PT endocytic function.[8,9] The lack of surface-bound and intracellular albumin likely reflects the experimental design, in which albumin was prebound to the cell surface on ice, then removed, and the cells warmed for 20 minutes. This approach significantly underestimates endocytic capacity in well-differentiated cells, where, as in native PT, a large fraction of majority of megalin and cubilin receptors are localized intracellularly at a steady state. Moreover, albumin dissociates rapidly from the cell surface after infinite dilution, further limiting the efficiency of endocytosis. In our study, OK cells internalize over 100-fold more albumin during a 15 minute incubation at 37°C than binds on ice.[5] In the mPTCs depicted, surface-bound albumin is not apparently distributed to the apical domain, and thus receptors other than megalin and cubilin may also contribute to the robust binding uptake observed in these cells.

Finally, the bromodeoxyuridine staining used to argue that the proliferation/differentiation balance in mPTCs is more tilted toward differentiation than the other lines appears to have be made using cells at different confluencies. Although HK-2 cells are difficult to culture to confluency, OK and LLC-PK1 cells readily form differentiated confluent monolayers that exhibit contact inhibition.

Just as rodent animal models fail to fully recapitulate human kidney physiology, there is no single PT cell line that perfectly replicates *in vivo* function. In addition to the transcriptomic analysis already available,[1] a comprehensive comparison of PT cell models should be expanded to include morphologic specializations, ion transport function, endocytic capacity, and metabolic considerations. We and others have also observed that specific culture conditions can contribute significantly to these outcomes. For example, megalin and cubilin expression and albumin uptake by many PT cell lines is increased by culture on permeable supports and by exposure

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to continuous orbital shear stress. In OK cells at least, the enhanced oxygenation and mechanosensitive stimuli provided by culture in this way also cause expansion of the apical endocytic apparatus, development of basolateral membrane invaginations, and a shift to oxidative metabolism.[3,4] That said, studies in OK and LLC-PK1 cells are challenged by the lack of cross-reacting antibodies and genetic tools, and may not adequately replicate human disease models. Informed consideration of appropriate models for studies on PT function will improve both the effect and rigor of *in vitro* experiments that complement and extend studies performed in humans and animals.

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