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Perspectives

PERSPECTIVES

The Mechanisms of Cellular Plasticity in Collecting Duct Cells: Intermediate Cell Type and Notch-mediated Transdifferentiation

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A Perspective on "Loss of Adam10 Disrupts Ion Transport in Immortalised Kidney Collecting Duct Cells"

It is well known that the cortical collecting duct segment of the aldosterone-sensitive distal nephron is composed of the predominant principal cells as well as type A, type B, non–type A, and non–type B intercalated cells. Importantly, previous studies determined that most intercalated cells, similar to principal cells, are descendants of $Aqp2^+$ cells.[1] While principal and intercalated cells are structurally different and easily identifiable, it is challenging to discriminate distinct subtypes of intercalated cells due to the great structural diversity among them. Moreover, under stressful conditions, such as metabolic acidosis, intercalated cells are adaptable and may convert between types A and B.[2] As was recently described using single-cell transcriptional profiling analyses,[3] the collecting duct may generate a spectrum of cell types via a newly identified transitional cell. This intermediate cell phenotype expressed markers for both intercalated and principal cells, suggesting cell types in the collecting duct may undergo cellular transitions modulated by environmental influences. It has been proposed that collecting duct cell plasticity is in part mediated by Notch ligand and receptor interaction. Cellular remodeling is likely directly related to abnormal cell populations in chronic kidney diseases; however, this process is still under investigation.

Over 15 years ago, Bernard Rossier's research group generated their immortalized mouse cortical collecting duct cell line ($mCCD_{cl1}$). This seminal paper allowed Gaeggeler et al.[4] to determine binding coefficients and receptor occupancy of the mineralocorticoid and glucocorticoid receptors in principal collecting duct cells. A key feature of these cells is their ability to form high-resistance monolayers with vectoral sodium and potassium transport that respond to a number of different hormones, including aldosterone, insulin, epidermal growth factor (EGF), and vasopressin at physiologically relevant concentrations. The combination of these features makes $mCCD_{cl1}$ cells a very attractive research model. Since then, numerous advancements in renal physiology have been published based on work done with $mCCD_{cl1}$ cells, including the study by Assmus et al.,[5] which was recently published in *Function*. In this study, the authors utilize $mCCD_{cl1}$ cells to study collecting duct cell plasticity. In a unique twist, they eliminated several factors that make $mCCD_{cl1}$ cells an attractive model in order to investigate signaling pathways involved in the transdifferentiation from principal to intermediate cells.

As mentioned above, the $mCCD_{cl1}$ cell line has been used to understand principal cell physiology on the basis of their transepithelial resistance and amiloride-sensitive electrogenic sodium transport. However, in their earlier study, Assmus and colleagues were able to show that the $mCCD_{cl1}$ cell line is not a homogeneous population of principal cells.[6] Similar to the results obtained from single-cell RNA-sequence profiling of mouse cortex tissue,[3] the $mCCD_{cl1}$ cells also exhibit features of intermediate and intercalated cells. Moreover, the authors revealed that plasticity is an intrinsic property of the $mCCD_{cl1}$

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cells, and this cell line can be used to precisely dissect mechanisms of cell differentiation in the collecting duct. We already know from research in animal models that different Notch signaling components, such as Foxi1 and Adam10, are critical for cellular plasticity and developmental cell fate determination in the collecting duct.[7] Foxi1 is a member of the forkhead box transcription factor family, which is essential for intercalated cell differentiation, and Adam10 is a secreted transmembrane metalloprotease that can activate Notch signaling through proteolytic cleavage. A previous study found that in mice lacking the transcription factor Foxi1, both principal and intercalated cells were replaced by cells with an intermediate phenotype.[8] Additionally, Adam10 deficiency in a mouse model was also shown to reduce the percentage of principal cells and increase the percentage of type A intercalated cells, further supporting the importance of the Notch signaling pathway in cell-type determination in the collecting duct during development.[9]

Using the CRISPR-Cas gene editing strategy to knockout Adam10 in $mCCD_{cl1}$ cells, Assmus and colleagues[5] demonstrated that loss of Adam10 results in significantly reduced amiloride sodium transport. Going further, the authors performed single-cell RNA sequencing to compare the transcriptomes of the knockout line to the original $mCCD_{cl1}$ line. Their data demonstrated strong cell remodeling with a significant decrease in β- and γ-subunits of ENaC, Claudin7, and transferrin receptor 1. As predicted, these changes indicated an increase in the percentage of intermediate cells expressing both principal and intercalated cell markers. Knockout of Adam10 also caused impaired cellular polarization, which may contribute to de-differentiation of cells and is particularly relevant to pathology given that loss of cell polarity and cell–cell adhesion is necessary for epithelial to mesenchymal transition.

The discovery of intermediate cells opens new avenues for our understanding of renal cellular plasticity in physiological, regenerative, and pathological processes, where the ability of the collecting duct cells to transdifferentiate and maintain homeostasis in adult tissues may play a key role. The known evidence and mechanisms for cell plasticity in the kidney and collecting duct are expertly summarized in a recent review, which provides a clear description of the often-confusing terms such as transdifferentiation, transdetermination, and self-renewal.[10] Plasticity is distinct from developmental pathways, although, frequently, there is overlap, such as how the loss of Adam10 reduced principal cell frequency during development and converted a predominantly principal cell line into a more intermediate collecting duct cell type. What is clear at this junction is that we still have a lot to learn about cell plasticity in renal disease and homeostasis, and, if we are lucky, sometimes the tools to understand emerging fields that are already part of our research history.

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