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See related article, "Impact of Solute Intake on Urine Flow and Water Excretion," on pages 1076–1078.

Integrins, Extracellular Matrix, and Terminal Differentiation of Renal Epithelial Cells

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The mechanism whereby epithelial cells terminally differentiate is an active area of investigation. One potential interface is the spatial and temporal expression of the transmembrane receptors known as integrins and the extracellular matrix (ECM) proteins to which they bind. In this issue of *JASN*, Vijayakumar *et al.*[1] propose that activation of integrin $\alpha v\beta 1$ causes synthesis and deposition of hensin, an ECM protein that forms 50- to 100-nm-long fibers composed of several fibrils. Upon polymerization and deposition into the ECM, hensin binds to $\alpha 6$-containing integrins, a key step in mediating the conversion of epithelial cells to a cuboid-like phenotype capable of apical endocytosis. These novel studies suggest that integrin–hensin interactions play an important role in the terminal differentiation of intercalated cells of the collecting duct.

The collecting system of the kidney is derived from the ureteric bud, which undergoes multiple iterations of branching morphogenesis followed by a phase of growth, maturation, and differentiation.[2] Many mechanisms regulate this branching and tubular expansion. Multiple transcription factors, growth factors, ECM proteins, and various cognate receptors play a critical role in these processes. Less information is available on the moieties that halt collecting duct growth and induce terminal differentiation. A number of transcription factors modulate the terminal differentiation of epithelial cells in

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various nephron segments. In this context, the DNA-binding protein/tumor suppressor p53 is one such factor, and its stabilization by phosphorylation and acetylation enhances the transcription of genes associated with terminal differentiation, including aquaporin 2 and the Na^+/K^+ ATPase.[3,4] In addition, p53 cooperates with at least two other transcription factors, cAMP response element-binding protein and Kruppel-like factor 4, to mediate its transcriptional regulation.[5] Another example of transcriptional regulation of collecting duct differentiation is the forkhead transcription factor Foxi1, which mediates differentiation of intercalated cells from precursor epithelial cells. When Foxi1 is deleted in mice, they exhibit impaired expression of intercalated cell–specific genes such as Pendrin, H^+-ATPase, and AE1, with no altered expression of principal cell markers.[6] Thus, transcriptional processes or factors/co-factors that regulate the nuclear chromatin surrounding transcription are critical for modulating the expression of genes responsible for induction and maintenance of a differentiated cell phenotype.

Among ECM proteins, hensin modulates terminal differentiation by favoring the conversion of β to α intercalated cells through a process that requires polymerization of hensin by galectin 3.[7,8] A key question is how hensin might regulate this terminal differentiation of intercalated cells. Although the answer is not clear, some clues are provided by Vijayakumar *et al.*[1] In this study, the authors show that polymerized hensin signals intercalated cells *in vitro* through α6-containing integrins. Although they were unable to delineate clearly which α6-containing integrin ($\alpha6\beta1$ and/or $\alpha6\beta4$) mediates this signaling, blocking anti-β1 antibody experiments suggest the hensin-mediated signal is integrin $\alpha6\beta1$ independent, which leaves integrin $\alpha6\beta4$.

Integrin $\alpha6\beta4$ is unique among the 24 known mammalian integrins, because the β4 subunit cytoplasmic tail is composed of 1017 amino acids and has distinctive cytoskeletal and signaling functions.[9] Furthermore, the β4 subunit is unique because it promotes the assembly of hemidesmosomes, specialized structures present in epithelial cells that create stable adhesion by connecting the intracellular keratin cytoskeleton to basement membrane.[10] Thus, $\alpha6\beta4$ not only performs a major adhesive function in cells but also promotes polarization, a final event in epithelial cell differentiation. The highly polarized and tight junction epithelial cells found within the medulla of the kidney certainly benefit from such structures. Although the best described ligand for integrin $\alpha6\beta4$ is laminin-332 (also known as laminin-5), it is possible that hensin is also a ligand for this receptor. Alternative explanations could be that hensin interacts with laminin-332, thereby enhancing its interaction with integrin $\alpha6\beta4$, or it might induce laminin-322 expression directly.

The mechanisms whereby integrin $\alpha6\beta4$ regulates the terminal differentiation of intercalated cells are purely speculative at present. Hemidesmosome formation in epithelial cells along the collecting duct might favor not only cell polarization but also resistance to apoptosis. In this context, nonpolarized cells are sensitive to induction of apoptosis, whereas polarized cells are resistant with ligation of the β4 integrin.[11] It is also conceivable that ligation of integrin $\alpha6\beta4$ by hensin induces the activation of specific integrin $\alpha6\beta4$-mediated signaling pathways, or the β4 subunit works as an "adaptor" for growth factor receptor signaling.[9] Both of these pathways might alter the transcription of genes required for the terminal differentiation of intercalated cells.

Although hensin is the first ECM protein shown to regulate terminal differentiation of intercalated cells within the collecting duct, it is highly likely that other ECM proteins along with basement membranes also regulate terminal differentiation of epithelial cells in different segments of the nephron and collecting system. Thus, the novel finding of Vijayakumar *et al.* suggests a critical role for the spatial/temporal expression of ECM proteins and their receptors in segmental renal function.

DISCLOSURES

None.

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See related article, "Role of Integrins in the Assembly and Function of Hensin in Intercalated Cells," on pages 1079–1091.