Commentary

Family Portrait

Renal Progenitor of Bowman's Capsule and Its Tubular Brothers

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As long ago as 1887, Podwyszożki¹ noted that extensive cellular regeneration could take place in the renal tubules after injury. In honor of the Kantian thought, he termed this phenomenon *regeneration per intussusceptionem*, suggesting that regenerating cells were "growing from within"¹ and that novel tubular cells could be derived from cells that survived. However, the most interesting and detailed description of tubular regeneration was published in 1928 by Hunter,² who reported that after injury, in adult human kidneys "the tubules are almost completely relined by a new and atypical cell differing very markedly from the original epithelium in size, shape and staining qualities ... The regenerated cells have the staining reaction of embryonic epithelium."² 465-466

The origin of these "atypical cells" in regenerating tubular epithelium was largely debated throughout the past century. In recent years, several studies have suggested that after injury, differentiated tubular cells proliferate and migrate to replace the neighboring dead cells.³ However, to explain the substantially modified phenotype shown by the regenerating tubular epithelium, these studies hypothesized that tubular cells underwent a process of "dedifferentiation."³ Indeed, in healthy adult human kidneys, renal tubular epithelial cells are columnar and cuboidal and express cytokeratin and adherens junctions that contribute to apical-basal polarity.³ After injury, regenerating tubular cells lose apical-basal polarity, show flattened and elongated morphologic features, and express the intermediate filament vimentin, thus acquiring a phenotype that is similar to those of the metanephric mesenchyme-derived renal embryonic progenitors.^{3,4} This indicates that the regenerative process is associated with a reversal to a less differentiated phenotype, fol-

lowed by redifferentiation, when the mesenchymal markers are lost, epithelial markers return, and physiologic function is restored.^{3,4}

In the current issue of *The American Journal of Pathology*, Lindgren et al⁵ propose an alternative hypothesis to explain the origin of atypical cells in regenerating tubular epithelium. Indeed, almost a century after Hunter's observations, these authors suggest the existence in adult human kidneys of rare embryonic-like tubular progenitors scattered among the more differentiated proximal tubular cells. Lindgren et al⁵ isolated from healthy adult human renal tissue a cell population displaying high aldehyde dehydrogenase activity, which is considered as a shared property of stem/progenitor cells in multiorgan systems.⁶ By gene expression profiling of the aldehyde dehydrogenase high and low cell fractions isolated and by subsequent immunohistochemical interrogation of renal cortical tissues, they delineated a rare cell population distributed in the epithelial layer of the proximal tubules in healthy adult human kidneys defined by coexpression of CD133 and CD24, two markers that selectively characterize the recently identified population of renal progenitors in Bowman's capsule (Figure 1).⁷⁻¹⁰ In adult human kidney, CD133⁺CD24⁺ renal progenitors of Bowman's capsule exhibit self-renewal potential, and they consist of a hierarchical population of progenitors that are arranged in a precise sequence in Bowman's capsule and exhibit heterogeneous potential for differentiation and regeneration.¹¹ Cells localized to the urinary pole that express CD133 and CD24 but not podocalyxin (PDX) or other podocyte markers (CD133⁺CD24⁺PDX⁻ cells) could regenerate tubular cells and podocytes. In contrast, cells

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Figure 1. Proposed mechanisms of tubular regeneration. $CD133^{+}CD24^{+}$ renal progenitors (red) are localized at the urinary pole and are in close contiguity with tubular renal cells (yellow) at the tubuloglomerular junction and with podocytes (green) at the vascular stalk. $CD133^{+}CD24^{+}$ tubular progenitors (orange) displaying mixed features of renal progenitors (red) and proximal tubular cells (yellow) localize at the tubuloglomerular junction and are scattered among more differentiated tubular cells. These tubular progenitors may proliferate and differentiate after tubular injury to replace dead cells. In contrast, $CD133^{+}CD24^{+}$ podocyte progenitors (red/green) display features of either renal progenitors (red) or podocytes (green) and localize between the urinary pole and the vascular stalk.

localized between the urinary pole and the vascular pole that expressed both progenitor and podocyte markers ($CD133^{+}CD24^{+}PDX^{+}$) could regenerate only podocytes.¹¹ Finally, cells localized to the vascular pole did not exhibit progenitor markers but displayed phenotypic features of fully differentiated podocytes ($CD133^{-}CD24^{-}PDX^{+}$ cells) (Figure 1).¹¹ More recently, genetic tagging provided the proof of concept that the renal progenitors of Bowman's capsule represent a hierarchical population of stem/progenitor cells also in rodents and that they can proliferate and progressively differentiate into podocytes from the urinary pole to the vascular pole.¹² However, evidence that $CD133^{+}CD24^{+}$ progenitors of Bowman's capsule can also regenerate tubular cells has currently been provided only in humans.^{7–11} Indeed, $CD133^{+}CD24^{+}$ renal progenitors purified by adult human kidneys regenerated tubular structures in different portions of the nephron and reduced the morphologic and functional kidney damage in SCID mice affected by acute tubular necrosis, sug-

gesting that these cells can participate in tubular regeneration in adult human kidneys.^{7–9} In addition, several previous studies have demonstrated that the proximal tubule arises at a variety of angles from Bowman's capsule and that at least one part of the tubuloglomerular junction has an area of intermediate appearance, with prominent microvilli on parietal cells in humans, mammals, and fish.^{10,13} These findings further confirm that renal progenitors of Bowman's capsule can, indeed, change into not only podocytes but also tubular epithelial cells, and it has been previously suggested that this might particularly occur as the kidney grows, during severe renal disorders,¹³ and during aging.¹³ The existence of a bipotent progenitor at the urinary pole of Bowman's capsule (Figure 1) is also in agreement with observations performed in adult exocrine glands, where adult stem cell compartments containing bipotent progenitors are usually localized at the point of connection between the lobule, which produces the secretion, and the duct, which modifies the secretion and collects it in the external of the gland.¹⁴ However, the duct of the exocrine gland is usually short, whereas the tubular part of the nephron is very long, which suggests that the stem cell compartment localized at the beginning of the tubular structure probably does not represent the unique source of regeneration of the tubular part of the nephron. The results of the study by Lindgren et al⁵ provide an intriguing novel hypothesis that rare $CD133^{+}CD24^{+}$ renal progenitors may localize in the tubule and proliferate and differentiate after tubular injury (Figure 1). Accordingly, tubular epithelium regenerating on acute tubular necrosis displayed long stretches of $CD133^{+}CD24^{+}$ cells, further substantiating that the atypical cells that are repairing tubular epithelium may simply represent the result of proliferation and differentiation of $CD133^{+}CD24^{+}$ tubular progenitors.

The strength of this hypothesis is further supported by the long list of markers that are uniquely shared by the scattered $CD133^{+}CD24^{+}$ tubular progenitors and their counterparts of Bowman's capsule but not by all the other epithelial cells of the nephron.⁵ Indeed, functional and bioinformatics analyses allowed a detailed characterization of the transcriptome of these cells, which indicated that they are endowed with a robust phenotype allowing increased resistance to acute tubular injury.⁵ More important, the renal progenitors of Bowman's capsule and their tubular counterparts are characterized also by expression of cytokeratin 7, cytokeratin 19, the antiapoptotic gene *BCL2*, the regeneration-promoting molecule MYOF, and vimentin, suggesting a unique phenotype⁵ characterized by coexpression of epithelial and mesenchymal markers, which has previously been reported for the renal progenitors of Bowman's capsule.⁶ Coexpression of cytokeratin and vimentin, and of the renal progenitor's markers *CD133* and *CD24*, is also a distinctive feature of the renal embryonic progenitor.^{13,15} Indeed, $CD133^{+}CD24^{+}$ cells also represent common progenitors of tubular cells and podocytes during kidney development,^{13,15} as shown by the observation that *CD133* and *CD24* coexpression identifies a subset of cells in the primordial nephron that display self-renewal and multidifferentiation potential.^{13,15} $CD133^{+}CD24^{+}$ progenitors

are located in condensed mesenchyme-derived primordial structures, primary vesicles, comma-shaped bodies, and S-shaped bodies.^{13,15} However, when the primordial nephron elongates and a primitive vascular tuft becomes evident in the cup-shaped glomerular precursor region, a cluster of CD133⁺CD24⁺ progenitors can be localized only at the urinary pole of Bowman's capsule.^{13,15} It is, however, possible that during the elongation process, which requires CD133⁺CD24⁺ proliferation and differentiation, some of the CD133⁺CD24⁺ cells that constitute the S-shaped body remain included as rare single cells in the maturing nephron and constitute the scattered CD133⁺CD24⁺ tubular progenitors.

The results of the study by Lindgren et al⁵ are also in agreement with those of lineage tracing experiments performed by Humphreys et al¹⁶ using transgenic mice to mark cells derived from the renal embryonic progenitors in adult kidneys. In this model, a fusion protein leads to constitutive and heritable expression of a marker gene such that all metanephric mesenchyme-derived renal epithelial cells, from Bowman's capsule to the junction of the connecting segment and the collecting duct, are heritably labeled.¹⁶ In contrast, the entire interstitial compartment and the collecting ducts remain unlabeled.¹⁶ After the induction of acute kidney injury, the repair of injured nephrons was accomplished by intrinsic cells localized in the cortical nephron, suggesting that repair of injured tubules is accomplished only by resident renal epithelial cells that represent the direct progeny of the renal embryonic progenitor.¹⁶ These results also demonstrated that the contribution of any stem/progenitor cell localized outside the cortical nephron for repopulation of the tubular epithelium is irrelevant. This is noteworthy because previous studies have suggested that progenitor cells with regenerating potential for renal epithelium could be located in the renal interstitium or among cells of the collecting ducts.^{8–10,17} The absence of label dilution after injury and repair also definitively demonstrates that bone marrow-derived cells do not directly contribute to renal repair.^{8–10,17}

Finally, the study by Lindgren et al⁵ provides another provocative observation, which is demonstration of the close relationship between the transcriptome of these tubular progenitors and the one derived by papillary renal cell carcinomas. This is further confirmed by the observation that the combination of cytokeratin 7, cytokeratin 19, and vimentin, which is a unique feature of CD133⁺CD24⁺ renal progenitor in the adult human kidney, is used by pathologists to discriminate papillary renal cell carcinomas from all other renal cell carcinoma subtypes.¹⁸ Papillary renal cell carcinomas are derived from cells of the proximal convoluted tubule similar to clear renal cell carcinomas, but they show a distinct immunostaining phenotype.¹⁸ Thus, these observations raise the provocative hypothesis that papillary renal cell carcinomas may directly derive from CD133⁺CD24⁺ renal tubular progenitors, whereas clear renal cell carcinomas may derive from other more differentiated proximal tubular cells. Accordingly, Lindgren et al⁵ provided evidence of strong CD133 expression by the papillary renal cell carcinomas analyzed. These results are also in

agreement with the concept that altered growth and/or differentiation of CD133⁺CD24⁺ renal progenitors may also be responsible for the generation of disorders.^{10,19} This concept is widely accepted in several stem cell systems, and it has recently been reported for the CD133⁺CD24⁺ renal progenitors of Bowman's capsule and for the CD133⁺CD24⁺ podocyte-committed progenitors, which can generate hyperplastic glomerular lesions of different types of podocytopathies and in crescentic glomerulonephritis and eventually lead to glomerulosclerosis.^{10,19}

Although the study by Lindgren et al⁵ provides convincing answers to many of the questions we had about tubular regeneration and opens a novel interesting hypothesis for the pathogenesis of some subtypes of renal cell carcinomas, there are several questions that still need to be answered. Direct proof of the regenerative capacity of these CD133⁺CD24⁺ tubular progenitors has to be provided using *in vivo* models of acute tubular injury. This will require the identification of novel markers that may allow distinction between the CD133⁺CD24⁺ renal progenitors of Bowman's capsule and those scattered in the tubular portion of the nephron and a direct study of the two populations, as has been performed for CD133⁺CD24⁺ podocyte-committed progenitors using the podocyte marker PDX.¹¹ This may allow us to also define the tubular lineage of the stem cell compartment of the kidney and to understand how it is generated and regulated. In addition, genetic tagging experiments are required to provide the proof of concept that this population participates in tubular regeneration after acute and/or chronic tubular injury. However, using transgenic mouse lines that express LacZ or Cre-recombinase under the control of an NFATc1 autoregulatory enhancer, a previous study already suggested the putative existence of a scattered progenitor cell population that proliferates to repopulate the proximal tubule after injury with HgCl₂.²⁰ This putative progenitor population is acutely resistant to apoptosis and subsequently participates in regeneration of the damaged proximal tubule in rodents.²⁰ Finally, further studies are necessary to evaluate the possibility that different subtypes of renal cell carcinomas may be generated by cancer stem cells that are directly derived from distinct stages of renal stem cell differentiation toward the tubular lineage.

The existence of a stem cell compartment and of putative tubular progenitors does not exclude the existence of further regenerative strategies to repair injured tubular cells in adult mammalian kidneys. Indeed, we have learned from planarians, fish, and amphibians, which exhibit high regenerative potential, that different strategies exist to regenerate injured organs and tissues. For example, working organ cells that normally do not divide can multiply and grow to replenish lost tissue, as occurs in injured salamander hearts.²¹ In addition, specialized cells can undo their training and assume a more pliable form that can replicate and later respecialize to reconstruct a missing part. Salamanders and newts take this approach to heal and rebuild a severed limb, as do zebra fish to mend clipped fins.²¹ Finally, pools of stem cells can step in to perform required renovations. Planaria tap into this resource when reconstructing themselves.²¹ Al-

though it is not yet clear how regenerative strategies that are observed during the early phases of evolution are applied in higher vertebrates, animals exploit different strategies for regeneration. Converging data suggest that usually more than one mechanism is involved in regeneration of a tissue^{10,13} because this confers an advantage during evolution. Further studies are needed to understand whether different mechanisms of regeneration contribute to tubular repair during acute and/or chronic renal disorders. However, the existence of a stem cell compartment suggests that novel treatments aimed at promoting the regenerative capacities of the kidney could conceivably be possible. Before that can happen, we will have to understand the signals that control the growth and differentiation of CD133⁺CD24⁺ renal progenitors.

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