From Patient to Dish and Back Again: Are We There Yet?

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Chronic kidney disease affects significant parts of the general population, and novel treatment strategies are warranted. In this issue, Lazzeri et al.1 explore the use of exfoliated cells in the urine of children affected by proteinuric diseases for diagnostic and therapeutic purposes (from patient-to-dish-to-patient). The authors investigate whether the isolated cells represent putative renal progenitor cells (RPCs) and test whether they are similar to a previously isolated putative progenitor population. They find that both cell populations ameliorate doxorubicin-induced nephritis in severe combined immunodeficiency mice. The controversial identity of the isolated cells and their potential use for diagnostic purposes or stem cells therapy are discussed.

Chronic kidney disease affects an estimated 5% of the general population. It represents a major risk factor for cardiovascular disease and increased mortality at least as potent as smoking or arterial hypertension. Glomerular diseases are still the most common causes of ESRD. It is time to translate recent advances in our understanding of glomerular diseases into novel improved diagnostic and therapeutic strategies.

Several approaches can be used. One is to search for unique intrinsic cells depending on specific signaling pathways, which can be manipulated by specific pharmacologic interventions in situ in vivo. Alternatively, renal cell populations can be isolated to then be used for diagnostic purposes. Moreover, isolated cells can be expanded in culture and/or manipulated to then be returned back into the diseased organism (i.e., from patient to dish to patient).

In this issue of JASN, this latter strategy was explored by Lazzeri et al.1 The authors established cultures of rare exfoliated cells from the urine of children affected by different proteinuric disorders of the kidney. The authors noted that cultured cells expressed a certain combination of markers (CD133–, CD24+, CD106+ [VCAM–1], and uroplakin III negative). In addition, cells coexpressing this combination of markers showed a higher proliferative index and expressed RNA transcripts similar to previously isolated cultures of adult parietal epithelial multipotent progenitor cells (APEMPs). The authors propose that the cultured cells represent the previously postulated fixed intrinsic population of progenitor cells (urinary renal progenitor cells [u-RPCs]).2 To substantiate this, the authors repeated an experiment where doxorubicin-induced renal disease in immunodeficient severe combined immunodeficiency mice was treated by intravenous injection of human APEMPs, u-RPCs, or cultured cells expressing other markers.3 Only the APEMPs and u-RPCs engrafted into the kidney and ameliorated proteinuria. In addition, the u-RPCs engrafted to regenerate podocytes and proximal tubule cells. Finally, the authors explored the tool of cultured u-RPCs for personalized investigations of genetic kidney disorders. They found that u-RPCs from patients with homozygous podocin mutations expressed lower levels of podocin. In a patient with a mutated LMX1B gene, authors found that filamentous actin distribution was altered in cultured u-RPCs. The authors concluded that u-RPCs represent an innovative tool to investigate genetic kidney disorders in more detail.

SEARCH FOR INTRINSIC PROGENITOR CELLS WITHIN THE KIDNEY

It is still controversial whether intrinsic progenitors exist within the kidney. Within the glomerulus, parietal epithelial cells (PECs) are probably the best candidate cells to regenerate the essential and limited pool of podocytes. PECs proliferate at a low rate and are localized within the same compartment next to podocytes and express markers, which some researchers use to define stem or progenitor cells (e.g., CD24 or a glycosylated isoform of CD133). Indeed, we have shown by cell fate tracking that cells of Bowman’s capsule migrate onto the glomerular tuft and later become podocytes in juvenile mice.3 This finding was confirmed by two independent studies.4,5 However, using the same technology of in vivo cell fate tracking, podocyte regeneration from cells of Bowman’s capsule was nonexistent in adult animals during aging or when inducing glomerular hypertrophy.4,5 Recent studies determining absolute podocyte numbers and density also show in humans that, should any regenerative mechanism for podocytes exist within the glomerulus, it cannot be very effective.6

IDENTIFICATION OF AN INTRINSIC LIMITED PODOCYTE RESERVE

So why are new podocytes recruited from PECs exclusively in juvenile mice? When analyzing the origin of recruited
podocytes in more detail, we found a distinct population of committed but undifferentiated podocytes on Bowman’s capsule close to the vascular pole.4 This podocyte reserve expressed synaptopodin and WT-1 and was directly labeled in transgenic mouse lines, which allow either podocytes or PECs to be directly labeled in vivo. In humans, this committed podocyte reserve is more obvious. Recent stereological investigations showed that this reserve contributes up to 20%–25% of the total podocyte pool in glomeruli undergoing physiologic growth in infants during their development in the first years of life.7

SEARCH FOR PROGENITOR CELLS IN THE PROXIMAL TUBULE

Within the tubule, cellular regeneration after AKI may occur from a fixed intrinsic progenitor population or from any surviving tubule cell. Recently, a putative progenitor population was described as a novel population of rare cells with a de-differentiated phenotype scattered throughout the proximal tubule (termed scattered tubular cells [STCs]).8 After AKI, these cells became more abundant and proliferated more than the surrounding ordinary tubule cells.8,9 Interestingly, STCs and PECs express a very similar set of markers, suggesting a common transcriptional program. The PEC transgenic mouse line recapitulated this program because it also labeled STCs with high sensitivity in the proximal tubule and allowed us to trace these cells in vivo.10 Our results showed that any tubular cell may transiently acquire the STC phenotype, which is a specific transcriptional program in response to virtually any tubular injury. We found no experimental support for STCs representing a fixed intrinsic progenitor population.

What does this mean in regard to the study of Lazzeri et al.? The authors use the markers glyCD133, CD24, and CD106 to identify and define RPCs. These markers are also part of the common transcriptional program and are expressed by both PECs and proximal tubular cells with the STC phenotype.10 When culturing urinary PECs and proximal tubular cells, one can expect that they will activate the common STC transcriptional program in response to stimulatory culture conditions as used by Lazzeri et al. (the authors used 20% FCS supplemented with a mixture of growth factors) so that many of them may acquire an RPC phenotype (i.e., coexpressing RPC markers, including CD24 and glyCD133). Further characterization of the cell populations isolated by Lazzeri et al. will therefore be required in future studies.

CELLULAR HOMING IN STEM CELL THERAPY

Several stem or progenitor cell populations have been injected into the circulation to deliver the cells to the actual target tissue. Unfortunately, most of these cells undergo rapid apoptosis within the vascular compartment.11 When injecting bone marrow-derived cells into the renal artery of mice, significant numbers could still be detected after 24 hours within the kidney, but the injected cells were virtually absent after 48 hours.12 Furthermore, when injecting mesenchymal stem cells, multipotent adult progenitor cells, bone marrow–derived mononuclear cells, or neural stem cells into the peripheral vein of rats, most cells were trapped in the lung and only a few cells reached the arterial circulation.13 Finally, it is controversial whether podocyte progenitors can traverse the glomerular basement membrane to reach Bowman’s space to engraf. Initial reports that this might be possible in rodents with a defective glomerular basement membrane (Alport mice) could not be confirmed in later studies.14 Lazzeri et al. report that approximately 8% of podocytes and 7% of proximal tubule cells were derived from injected RPCs in a mouse nephritis model induced by doxorubicin. Considering that a total of 1.5 million cells were injected into the peripheral vein, undergoing the first-pass effect in the capillary network of the lung, and considering that a mouse has about 1 million podocytes and at least 20 times more proximal tubule cells, more than approximately 1 million cells apparently engrafted into the kidney. This can be explained either by an unusually small loss of cells to other organs and apoptotic cell death and a highly efficient homing into the kidney and/or by continued proliferation of injected cells without significant loss of the label. Alternatively, the lipophilic dye PKH26 used to label the outer membrane–injected cells was only a reversible staining, which can be released from injected cells and taken up by surrounding cells. Furthermore, PKH26 staining tends to be diffuse within complex tissue; therefore, the absolute number of cells can be overestimated. More experiments using irreversibly tagged cells are required to verify whether cells injected into the peripheral circulation engraf into the renal epithelial cell compartment.

SAFETY ISSUES

Injection of modified multipotent cells into a different compartment, such as blood circulation, may provoke potentially undesired effects. For example, we have shown that mesenchymal stem cells injected into the renal artery of rats maldifferentiated into adipocytes within glomeruli.15 In humans, injected stem cell preparations transformed into a teratoma-like growth.16

USE AS DIAGNOSTIC TOOL

The most imminent application of the protocol by Lazzeri et al. might be the use “for functional studies of potentially pathogenic mutations of unknown significance to complement the diagnosis of genetic kidney disorders,” as stated by the authors. In fact, the established protocol opens the possibility to investigate in a systematic fashion a multitude of unresolved questions. The authors mention two important examples: (1) it is
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still unclear if specific patterns of actin fiber distribution in cultured cells correlate with glomerular disease and (2) mutations of unknown significance can be investigated for their functional relevance in vivo in SCID mice carrying engrafted u-RPCs of the respective patient. Presently, these are still academic investigations. Patients and parents will have to be patient in the hope that one day these important innovations will translate into health benefits.

DISCLOSURES

None.

REFERENCES


