Regrow or Repair: Potential Regenerative Therapies for the Kidney

Melissa H. Little

Institute for Molecular Bioscience, University of Queensland, St. Lucia, Queensland, Australia

Regenerative medicine is being heralded in a similar way as gene therapy was some 15 yr ago. It is an area of intense excitement and potential, as well as myth and disinformation. However, with the increasing rate of end-stage renal failure and limited alternatives for its treatment, we must begin to investigate seriously potential regenerative approaches for the kidney. This review defines which regenerative options there might be for renal disease, summarizes the progress that has been made to date, and investigates some of the unique obstacles to such treatments that the kidney presents. The options discussed include *in situ* organ repair *via* bone marrow recruitment or dedifferentiation; *ex vivo* stem cell therapies, including both autologous and nonautologous options; and bioengineering approaches for the creation of a replacement organ.

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Regenerative Approaches to Renal Disease

The term *regenerative medicine* straddles cell biology, matrix biology, and bioengineering with the objective to regrow or repair a damaged organ or tissue type. It can be defined as the use of cells for the treatment of disease and encompasses both organ repair and the *de novo* regeneration of an entire organ (Figure 1). Organ repair can be delivered *in situ* or *ex vivo*. The simplest and most pharmacologically attractive strategy for organ repair *in situ* is the delivery of a soluble reparative factor that improves the ability of the kidney to repair itself. Although such an approach may involve the understanding of the factors that are produced by stem cells, this is not a cellular therapy and is not dealt with in this review. Other *in situ* possibilities include the recruitment of stem cells to the kidney to elicit repair and the induction of dedifferentiation of resident renal cells. Whereas some regard *in situ* approaches as more likely to be successful for an architecturally and anatomically constrained organ such as the kidney, the other approach is the *ex vivo* culture of stem cells for redelivery to the damaged kidney. This might involve autologous or nonautologous stem cells from a variety of sources. Finally, a bioengineering approach that relies on cells, factors, and matrix may be achievable. Although seemingly the most difficult, it may be the more feasible approach for genetic conditions such as polycystic kidney disease. The matrix of options illustrated in Figure 1 could be drawn up for almost any organ. This review investigates each option and relates it to the function and the structure of the kidney so as to examine its feasibility and identify the key obstacles to delivery.

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Address correspondence to: Prof. Melissa H. Little, Institute for Molecular Bioscience, Queensland Bioscience Precinct, University of Queensland, St. Lucia, Brisbane, Qld, 4072, Australia. Phone: +61-7-3346-2054; Fax: +61-7-3346-2101; E-mail: m.little@imb.uq.edu.au; Web: www.imb.uq.edu.au

Setting the Stage: Normal Kidney Development and Regeneration in Vertebrates

Regenerative biology draws on an understanding of normal developmental processes. Understanding the molecular basis of kidney development will be the key to the development of regenerative therapies for chronic renal disease. During mammalian development, three separate excretory organs develop: The pronephros, the mesonephros, and the metanephros. In mammals, it is the paired metanephroi that persist postnatally and constitute the permanent kidney. The permanent kidney arises *via* reciprocal interactions between two tissues, the ureteric bud (UB) and the metanephric mesenchyme (MM), the latter arising from the intermediate mesoderm (IM) (1). The UB gives rise to the collecting ducts and the ureter. The MM, which shows much broader potential and gives rise to all other elements of the nephrons, the interstitium, and the vasculature, is regarded as the renal progenitor population (2). As the UB reaches the MM, signals from the tips of the branching UB induce areas of adjacent MM to aggregate and undergo a mesenchyme-to-epithelial (MET) transition. Each MET event represents the birth of a new nephron with the first nephrons "born" in the center of the MM. The peripheral MM, which has not yet undergone induction, is referred to as the nephrogenic zone. Nephrogenesis in humans is complete by week 36 of gestation (3), whereas it continues for 1 to 2 wk after birth in the mouse and the rat. At that time, it is assumed that the peripheral nephrogenic zone is exhausted.

Can the kidney regenerate? In simple vertebrates, including fish and amphibians, metanephroi do not form and the permanent excretory unit is the mesonephros. Elasmobranchs (sharks, rays, and skates) constitute a unique example of "kidney" regeneration; their mesonephroi can undergo accelerated nephrogenesis after partial ablation to replace the missing parts (4). In the mammal, partial nephrectomy stimulates hypertrophy of remaining tissue, even in the contralateral kidney, but

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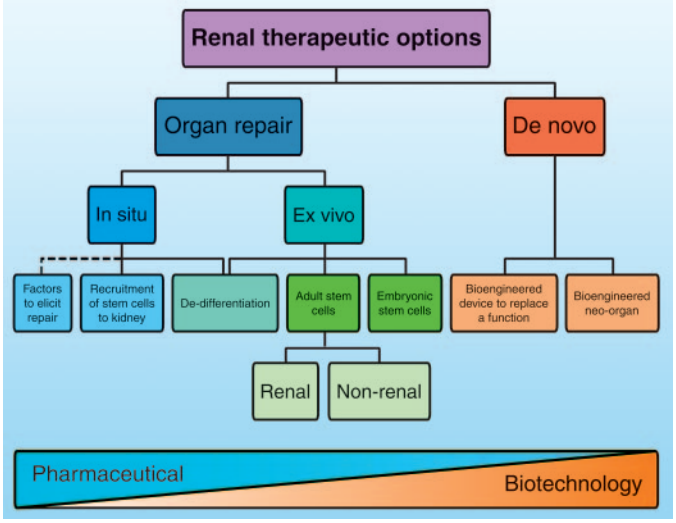

Figure 1. Potential therapeutic options for the treatment of renal disease. The options are presented as predominantly pharmaceutical to predominantly biotechnological from left to right. Illustration by Josh Gramling—Gramling Medical Illustration.

not the generation of new nephrons (5). However, whereas the resection of an adult kidney does not lead to the regeneration achieved in the liver, the mammalian kidney shares with the majority of organs the ability to repopulate and repair structures that have sustained some degree of injury. This process, termed *cellular repair*, can be achieved by reentry into mitosis and proliferation of neighboring cells. As a result, the kidney can undergo significant remodeling in response to acute damage. For example, obstruction of the ureter can result in the near destruction of the kidney medulla, but once the obstruction is removed, there is a rapid process of reconstruction and repair that will regenerate the tubules of the medulla without forming new nephrons (6). It has been proposed that the cells that elicit such repair come from interstitial cell transdifferentiation (7), tubular cell dedifferentiation and migration into the areas of damage before redifferentiation (8,9), the recruitment of stem cells from the bone marrow (10–14), or the generation of new tubular cells from an endogenous renal stem cell population (reviewed in reference [15]). Which of these is primarily responsible for the *cellular repair* that is observed after acute damage has not been proved definitively using lineage tracing. However, the mammalian kidney seems to have a very limited potential for *structural repair* or true regeneration. While nephrogenesis is occurring in the fetus, there is evidence that a systemic humoral response to nephrectomy allows the enhanced nephrogenesis of the remaining organ (16). However, nephrogenesis in mammals ceases just before or shortly after birth (3), and the birth of new nephrons has never been reported after this point in time. Chronic injury of the kidney, which is responsible for the majority of cases of end-stage renal failure, results in irreversible glomerular and tubular damage and resultant loss of renal function. Hence, mammalian kidneys respond to chronic damage by fibrosis, scarring, and irreversible functional loss.

Recruitment of Bone Marrow to the Kidney

Can we improve the capacity of the kidney for cellular repair? The ability of cells that originate from bone marrow to move into distant sites within the body, including the kidney, is now well recognized. Reports have suggested that these cells can transdifferentiate into tubular epithelial cells (12), mesangial cells (11,13,14), glomerular endothelial cells (17,18), and even podocytes (12). As in most organs, bone marrow–derived cells (BMDC) appear in the kidney in response to damage. The lineage of these cells is unclear, and their ability to elicit transdifferentiation is controversial because the possibility of cell fusion has not always been eliminated (19) (Figure 2). The use of lineage tracing has been critical to differentiating these two possibilities. In the case of the muscle, there is evidence from studies in which bone marrow was derived from LysM-Cre mice that it is the monocytic lineage that is recruited and fuses with cells in the target organ (20). This lineage gives rise to the macrophages, which express proteins that are involved in fusion processes. This does not answer the question of the relative value of this fusion process. In the brain, BMDC can fuse with Purkinje cells (21), a cell type that is presumed to be unable to divide, possibly leading to a "rejuvenation" of such terminally differentiated cell types. Certainly, the functional outcome of BMDC recruitment must always be assessed.

In the context of the kidney, several studies have examined the recruitment of BMDC to kidney in response to damage signals and their transdifferentiative and reparative capacity. The injury models used include ischemia-reperfusion injury

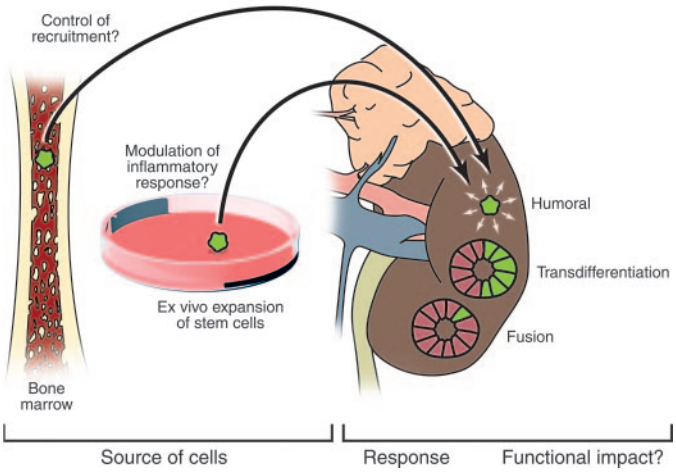

Figure 2. Stem cells, whether recruited to the kidney from distant organs or delivered to the kidney after *ex vivo* expansion of an isolated stem cell population, may contribute to repair *via* the production of specific cyto/chemokines or growth factors (humoral response), transdifferentiation into specific renal cell types, or cell fusion. It is not always clear which of these events occurred or which event was of the greatest functional significance. Although it has been shown to occur, the regulation of stem cell recruitment to the kidney has not been elucidated. There is increasing evidence for a humoral reparative role being provided by introduced stem cells, but the nature of this response also remains to be investigated. Illustration by Josh Gramling—Gramling Medical Illustration.

(22), folic acid–induced acute tubular injury (23,24), unilateral ureteric obstruction (25), and anti-Thy1 antibody–mediated glomerulonephritis (13). Bone marrow transplantation into HIgA mice, which have glomerulonephritis, improved renal function in these mice (26). In the studies in which careful quantification of recruitment to the tubular epithelium has been performed, donor-derived bone marrow has contributed between 0.06 and 11% of the epithelial cells (22–24). This level does decline with time. An initial recruitment level of 11% dropped to 0.67% at 28 d after ischemia with a concomitant increase in recruitment to the interstitium (22). Two seminal papers in this area (22,23) disagreed on whether there was evidence for transdifferentiation, but both concluded that while BMDC recruitment occurs, repair is predominantly elicited *via* proliferation of endogenous renal cells. Duffield *et al.* (23) maintain that BDMC contribute a regenerative cytokine environment that may be important in the resulting functional repair (Figure 2). If this process could be recapitulated pharmacologically, then repair may occur without the need for recruitment. Pretreatment of animals with stem cell factor and granulocyte colony-stimulating factor (granulocyte CSF) has been shown to improve recovery from ischemic injury in the absence of transdifferentiation of BMDC (27), and the combined pretreatment with granulocyte CSF and macrophage CSF provides renoprotection from cisplatin-induced renal failure (28). It also may prove valuable to improve recruitment. Held *et al.* (29) used a genetically induced model of chronic tubular damage that involved hereditary tyrosinemia (mutations in fumarylacetoacetate hydrolase) and mutations in homogentisic acid dioxygenase and reported significant integration (50%) of introduced BMDC. Hence, a drive for the selection of wild-type cells considerably increases the regeneration process (29). More recently, recruitment and apparent podocytic transdifferentiation of male BMDC to the glomeruli of mice that lacked collagen4α3 has been reported (30). This is a model of Alport syndrome in which there is considerable shedding of protein through the damaged glomerular basement membrane. Whereas podocytes have not been a reported site of bone marrow recruitment in other experimental models, this study claimed a bone marrow origin for 10% of the podocytes in these mice with a reduction in protein shedding and evidence of collagen replacement within the basement membrane. In this case, access may have been increased as a result of the altered permeability of the basement membrane, but BMDC from mutant mice were not recruited to the glomeruli of mutant recipients, suggesting an active selection for collagen-producing cells. In all of these reports of bone marrow recruitment to damaged kidneys, the lineage of the BMDC that were recruited has not been established. However, adoptive transfer of macrophages into a model of unilateral ureteric obstruction significantly reduced fibrosis in the late stages of this damage state (25). This may have involved transdifferentiation or an altered immunologic response. What also has not been investigated is whether the recruitment of BMDC is good or bad in cases of chronic renal damage.

Controlled Dedifferentiation as a Treatment of Renal Disease

Can we repair a kidney by recapitulating development? Among vertebrates, certain amphibians show a unique ability to regenerate completely complex organs or body parts (31). Salamanders, newts, and axolotls can reconstitute various anatomic structures such as limbs, spinal cord, heart, tail, retina, lens, and upper and lower jaws. In the case of the limb, this process involves dedifferentiation (*i.e.*, loss of a specialized phenotype to return to a progenitor phenotype), proliferation of the resulting primitive blastema, and then redifferentiation of cells in the vicinity of the injury (32) as opposed to the mobilization of a stem cell population *per se*. Muscle fibers, Schwann cells, periosteal cells, and cells from the connective tissue undergo dedifferentiation and then organize a blastema from which the new limb arises (Figure 3A). Can this be applied in higher vertebrates? Regeneration within the skate mesonephros is a process that takes place in an identified nephrogenic zone using a persistent field of progenitors that can be recruited for regeneration (Figure 3B). Whether these progenitors represent stem cells, as defined as a long-term, self-renewing cell population, has not been established. In mammals, there is no persistent blastema in the adult (Figure 3C). In the absence of such a persistent population of renal progenitors,

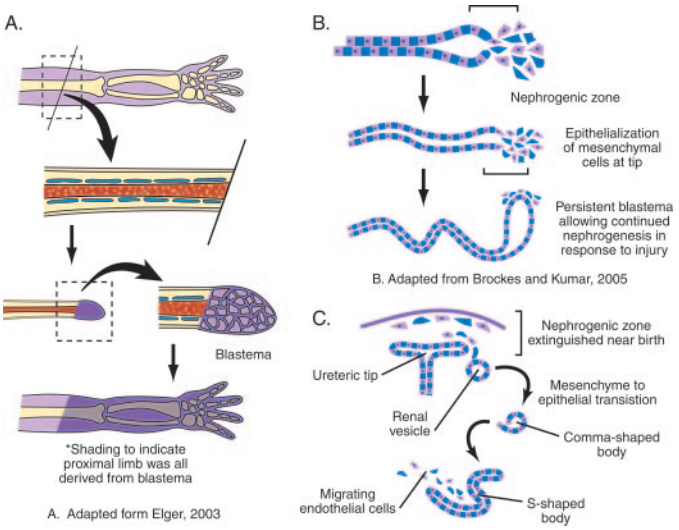

Figure 3. Different approaches to regeneration and repair within vertebrates. (A) Regeneration in the salamander limb in response to resection involves the dedifferentiation of muscle, bone, and connective tissue elements to form an undifferentiated mitotic blastema. This blastema re-patterns and re-differentiates into a limb equivalent only to the region that has been resected. (B) The development of nephrons within the skate mesonephros involves an incorporation into the end of the mesonephric tubules. The maintenance of a persistent blastemal population allows for structural repair *via* continued nephrogenesis. (C) Development of nephrons in mammalian metanephroi utilizes a mesenchyme-to-epithelial transition from an exhaustible nephrogenic zone, preventing "structural" regeneration after the cessation of nephrogenesis. Illustration by Josh Gramling—Gramling Medical Illustration.

could such a blastemal field be generated *via* dedifferentiation in the mammalian kidney? In a recent review of the obstacles to limb regeneration in the mammal (33), it was observed that mammalian limb cells lack the response of reentry into S-phase in response to thrombin (even though this response still would be present if a mouse cell were fused with that of a salamander), and their more complex immune systems respond to damage *via* the production of fibrosis and the recruitment of inflammatory cells. Possibly as a result of these differences, the production of the blastema that is required for regeneration does not occur, yet there are examples of cell types even in humans that show enormous regenerative capacities, together with more salamander-like properties such as an ability to recommence cell division and dedifferentiate to regenerate. Oligodendrocyte precursor cells have been reverted to multipotential neural stem cells that are able to proliferate and to give rise to neurons, astrocytes, and oligodendrocytes (34). More striking, highly specialized multinucleated muscle cells have been induced to dedifferentiate into mononucleated multipotent progenitor cells that are able to adopt the osteogenic, chondrogenic, adipogenic, and myogenic fates (35). In this case, the dedifferentiation was induced by ectopic expression of the transcriptional repressor Msx1 in combination with growth factor stimulation. Finally, the mouse MRL strain has been shown to have both a marked capacity not to scar and to restore normal myocardial tissue without scarring through a process the authors describe as similar to regeneration in amphibians (36). How feasible is dedifferentiation as a therapy? Postnatal cell turnover in the kidney has never been examined thoroughly, but the cellular complexity of this organ suggests that a dedifferentiation into blastema followed by redifferentiation for the purposes of regeneration would need to be as complex as that seen in the salamander limb. Hence, we need to understand the blastemal progenitors that give rise to the kidney and to understand the process that long has been observed in the kidney in response to short-term local damage: The epithelial-to-mesenchymal transition of tubular cells. If able to be induced, then dedifferentiation might be evoked *in situ* or *ex vivo* (Figure 4). *In situ* dedifferentiation would require controllable gene therapy to ensure a cessation of dedifferentiation and subsequent induction of regeneration, or it runs the risk of generating blastemal expansions as for a Wilms' tumor.

Stem Cells and Stem Cell–Based Therapy

Can we elicit cellular repair in the kidney *via* the introduction of stem cells? The development of stem cell therapies for kidney is in its infancy primarily because of the complexity of the organ involved, the degree of damage present at the time of diagnosis, and the belief that kidney development ceases at birth. Three sources of stem cells can be envisioned in the development of such treatments: (*1*) Renal adult stem cells, (*2*)

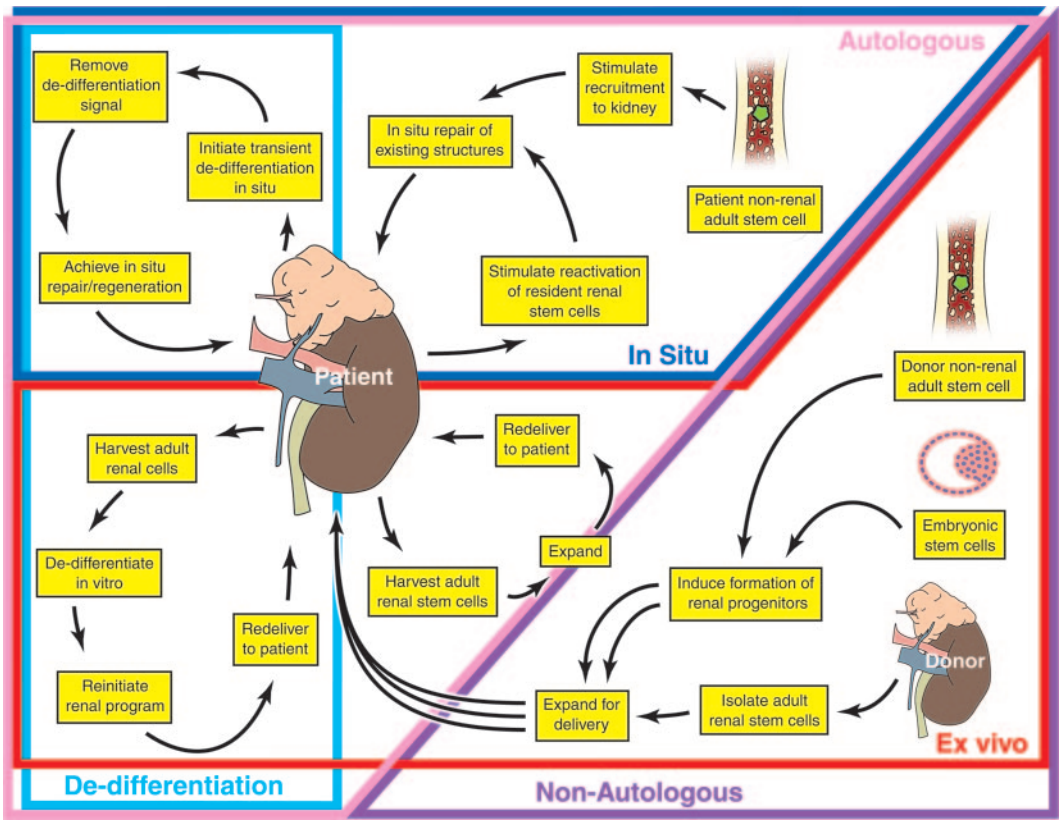

Figure 4. Cellular therapeutic options for the treatment of renal disease include *in vivo* and *ex vivo* options and may utilize autologous or nonautologous stem cells or the dedifferentiation of mature adult renal cells. These options currently are hypothetical. Illustration by Josh Gramling—Gramling Medical Illustration.

nonrenal adult stem cells, and (*3*) embryonic stem (ES) cells. These options are depicted in Figure 4.

Renal Adult Stem Cells

The existence of multipotent adult stem cells that are critical for the ongoing turnover of the skin, bone marrow, stomach, intestine, and cornea have been known for a long time. There now is strong evidence for the existence of adult stem cells with a much greater degree of plasticity in many organs. The derivation of cells that display apparent pluripotency has now been reported from many adult organs, including brain, bone marrow, skin, and fat (37–41). Such observations suggest that "stem cells" exist in all adult tissues. What does a renal adult stem cell look like, and where is it? Although many attempts have been made to identify such a population, no definitive data to date establish the existence of a long-term, self-renewing cell population with the capacity to generate distinct daughter cells with renal potential in the adult kidney. However, many approaches have been taken to look for such a population. Burrow and Wilson (42) reported the culture of nephrogenic zone cells from the developing human kidney in media from a Wilms' tumor cell line. They termed these cells *nephroblasts*. The critical components of the conditioned medium were never identified, and a similar cell type has never been cultured successfully from postnatal kidney. Kitamura *et al.* (43) screened for stem cell potential in various regions of the postnatal murine kidney *via* dissection of various nephron segments and culture after dissociation to single cell. In this way, they defined a cell line that was derived from the S3 segment of the proximal tubules. This could be maintained long term without transformation and expressed Pax2, Wnt4, and WT1. These cells seemed to contribute to renal tubules in a model of ischemia/reperfusion injury, but improvement in renal function was not assessed. Evidence of clonogenic self-renewal was not presented. With the increasing amount of literature on the expression profile of the developing kidney across time and subcompartment, it may become possible to dissect better the compartments, such as the nephrogenic zone and the cap mesenchyme, and identify cell surface marker combinations with which to search for a persistent fetal renal progenitor. In an attempt to define the profile of a renal progenitor population, expression profiling of the 10.5 d postcoitus mouse MM *versus* adjacent IM was performed (44). This identified the specific expression of transmembrane proteins such as CD24a and cadherin11 that differentially marked the MM at that time point. It remains to be shown whether a $CD24a^{+}cadherin11^{+}$ population persists in the adult kidney, whether such cells self-renew, and whether they show any renal capacity when isolated from adult tissue. On the basis of reports that CD133 marked hematopoietic stem cells/endothelial progenitors (45), Bussolati *et al.* (46) isolated $CD133^{+}$ cells from the adult kidney to examine their potential as renal stem cells. These cells did not seem to be derived from the blood ($CD34^{-}CD45^{-}$), did express some mesenchymal stem cell (MSC) markers ($CD29^{+}$, $CD44^{+}$, and $CD73^{+}$), but showed only limited differentiation capacity. However, they did express Pax2, homed to kidneys that were damaged *via* intramuscular glycerol injection, and gave rise to endothelial and tubular epithelial cells within these kidneys. The clonogenicity of these cells was not established, but the authors hypothesized that these interstitial cells could act as a supply of replacement tubular cells or assist in revascularization after damage.

One way of looking for stem cells in solid organs is a short administration (pulse) of bromodeoxyuridine (BrdU) followed by a long chase period. This approach is based on the premise that stem cells cycle slowly and, having incorporated BrdU into their DNA, will retain this label for a long time. Several groups have used this approach to identify slow-cycling cells in the kidney, which may represent renal stem cells. The timing of the pulse, duration of the pulse, and length of the chase are important to the interpretation of the results. Maeshima *et al.* (47) identified BrdU-labeled cells in the renal tubules, which they termed renal progenitor-like tubular cells. These cells reenter mitosis in response to renal damage and turn into fibroblasts (48). However, they also show the potential to become proximal tubule and collecting duct cells and can form tubular structures *in vitro* when cultured in collagen gel (49). The timing of the pulse (postnatal) and the length of the chase (2 wk) suggest that these cells are either differentiated tubular cells or tubular progenitors of limited potential. Indeed, careful immunohistologic analysis of cortical BrdU-labeled tubular cells suggests that these cells are unlikely to represent stem cells because they are identical to the surrounding terminally differentiated renal tubular cells (50). Oliver *et al.* (51) identified a population of BrdU label–retaining cells within the papilla of the kidney. BrdU was pulsed during the first postnatal week, during which nephrogenesis is continuing in the rodent, and the chase endured for 2 mo, indicating long-term label retention. These cells can be cultured under hypoxic conditions to form aggregates of nestin-positive cells, a marker in other stem cells types. In response to an ischemic insult, these cells seemed to reenter mitosis rapidly, particularly within the outer medulla. Although more likely to represent stem cells, clonogenicity was not established and these cells also may represent a transiently amplifying cell population that is recruited during injury rather than true stem cells. Definitive markers for the isolation of these cell types still are required. The above research justifiably has generated a great deal of excitement but no rigorous proof of the existence of a pluripotential adult renal stem cell with long-term self-renewal capacity and clonogenicity.

The ability of hematopoietic stem cells to efflux dyes such as Hoechst 33342 and Rhodamine 123 has been used as the basis of a single-step hematopoietic stem cell (HSC) isolation protocol (52). The term *side population* (SP) is used to describe HSC that are isolated in this way. The observation of cells with the same efflux profile in solid organs has raised the possibility of organ-based SP, which also may represent stem cells. Several groups have reported the existence of an SP in the adult rodent kidney (53–57). These data remain contradictory in terms of the relative size, origin, and lineage capacity of the renal SP. Iwatani *et al.* (55) showed no evidence for a capacity to transdifferentiate into renal cells *in vivo*. Hishikawa *et al.* (54,56) reported that these cells possess renal and multilineage capacity in culture. When assessing the effect of renal SP cell introduction into a model of renal damage, they observed little evidence for

transdifferentiation, with SP cells being located in the interstitium of the recipient kidney. Challen *et al.* (57), using more stringent isolation procedures, demonstrated that the renal SP represented 0.1% of the kidney. These cells showed an immunophenotype that was distinct from that of bone marrow SP, and these cells demonstrated evidence for multilineage potential *in vitro* and *in vivo*. However, despite stringent selection, this population remains heterogeneous with evidence of a monocytic fraction, which may result in the apparent phenotypic plasticity. In both studies (56,57), the introduction of SP cells into a model of acute experimental renal damage was reparative, suggesting a paracrine role for this cell type that, if characterized, may obviate the need for a cell at all. While SP cells from the bone marrow do represent HSC, it is not correct to assume that dye efflux activity alone indicates self-renewal capacity (58). No studies of the renal SP have proved self-renewal. Their reparative activity nevertheless warrants further investigation, and the definition of a marker phenotype that allows isolation without the assessment of dye efflux is needed. Musculin/MyoR has been reported as a marker of renal SP cells (54), but our own data do not support this (57), raising the question of SP homogeneity and the reproducibility of current isolation techniques.

Nonrenal Adult Stem Cells

With an increasing number of adult cells with seeming pluripotency, can these cells be encouraged to turn into renal cell types and assist in the treatment of renal damage? This review concentrates on the MSC. The term MSC refers to an adult stem cell that is present in the bone marrow in low numbers and has a capacity to differentiate into a wide range of mesenchymal tissue types, including cartilage, bone, muscle, stroma, fat, tendon, and other connective tissues. This term more recently has been applied to plastic adherent fibroblastic cells that are isolated from the bone marrow and other tissues that show mesenchymal multipotency. Few definitive markers identify these plastic-adherent mesenchymal cells, and in most studies, there is little definitive proof that these cells are true "stem cells." Hence, it has been proposed that these should be referred to more properly as multipotent mesenchymal stromal cells (59), although the acronym MSC can apply to both. Unlike HSC, once isolated, these mesenchymal stromal cells can be grown in culture for many population doublings and now have been shown also to have a much broader potential, including neural differentiation. In some studies, the surface phenotype of an MSC has been investigated. They are negative for markers that include CD34, CD45, and CD14 and positive for CD166, CD105, CD29, and CD44 (60). Several groups have now investigated the effect of delivery of MSC in models of acute renal damage. Herrera *et al.* (61) induced damage using an intramuscular injection of glycerol in C57/BL6 mice and monitored the fate of green fluorescent protein–MSC that were introduced intravenously. These cells homed selectively to damaged kidneys and seemed to differentiate into tubular epithelial cells. There also seemed to be evidence for a trophic role as shown by an increase in proliferating cell nuclear antigen–positive tubular cells throughout the kidney. A reduction in creatinine levels in the green fluorescent protein–MSC–treated mice suggested that a functional improvement was elicited by MSC. Morigi *et al.* (62) investigated the renoprotective capacity of MSC using a cisplatin model for acute renal damage and tracing the introduced cells using Y-chromosome fluorescence *in situ* hybridization. They also reported MSC integration into tubular epithelium and evidence for increased tubular proliferation that was not elicited by the introduction of HSC. More recently, another group using the ischemia/reperfusion model of acute damage in the rat also showed evidence for improved renal function after infusion of MSC. They attributed this renoprotective effect to a paracrine mechanism, because there was no evidence of transdifferentiation (63,64). MSC were iron dextran–labeled and then tracked using magnetic resonance imaging, suggesting that they were located primarily in the glomerular capillaries, as might be expected after an intravenous infusion. In light of their ability to be cultured; their ready accessibility from blood; and their apparent homing, transdifferentiation, and paracrine protective activities, this seems to be a very strong candidate for use as an adult stem cell in the treatment of renal disease. A greater understanding of the factors that regulate their homing and renoprotective activity may prove equally fruitful in delivering a cell-free approach to renal disease. To this end, Luttichaux *et al.* (65) have begun to define the chemokine receptors that are expressed by MSC *in vivo* and in culture. Togel *et al.* (64) highlighted a reduction in the production of proinflammatory cytokines and a concomitant increase in anti-inflammatory cytokines such as IL-10, TGF-α, Bcl2, and basic fibroblast growth factor after infusion of MSC into an ischemia/reperfusion model of acute damage.

Human ES Cells

The concept of stem cell–based therapy has grown rapidly since the derivation of human ES cells (hESC). ES cells are pluripotent cells that are derived from the inner cell mass of a developing embryo and have the capacity to divide indefinitely while retaining a pluripotent phenotype. Excitement about the potential to use ES cells to repair or regrow organs has increased since the derivation of ES cells or embryonic gonadal stem cells from human tissue (66–68). What potential is there for ES cells to develop into renal progenitors? Although more difficult to maintain and propagate than their murine counterparts (69), hESC have the ability to develop along ectodermal, mesodermal, and endodermal lineages. Cells of mesodermal origin are found in spontaneously differentiating cultures of hESC, and hESC now can be induced readily to undergo sequential hematopoietic (70) and cardiomyocyte differentiation under the control of members of the TGF-β superfamily (reviewed in reference [71]). This potential to derive mesodermal tissue bodes well for renal differentiation. The introduction of undifferentiated ES cells into a tissue usually results in teratoma formation. Yamamoto *et al.* (72) used this approach to provide evidence that murine ES cells had the potential to give rise to mesonephric ducts and UB in teratomas. In contrast, Steenhard *et al.* (73) reported 50% integration of undifferentiated ES cells into the tubules of embryonic kidneys without evidence for teratomas. Kobayashi *et al.* (74) created Wnt4-

transformed murine ES cells and showed *in vitro* that these had the capacity to form aquaporin 2–positive renal tubules. Kim and Dressler (75) sought to direct renal differentiation of murine ES cells by relying on previous research on commitment to early IM in *Xenopus* (76). They demonstrated that murine embryoid bodies (EB) that were cultured in a combination of activin A and retinoic acid expressed a number of markers of IM (Eya1 and Lim1), early kidney development (Pax2, WT1, Wnt4, Six2, and GDNF), and renal tubule–specific markers (cadherin-6) *in vitro*. Using lacZ to trace their progress, EB that were primed with retinoic acid, activin A, and bone morphogenic protein 7 when injected into embryonic kidney cultures showed 100% incorporation into developing renal tubules. There was no evidence that this process was occurring *via* cell fusion, because not a single fusion event was observed when an ES cell line that contained a loxP-flanked EYFP construct in the Rosa26 locus was injected into kidney explants that were isolated from a mouse line that expressed Cre recombinase in the developing renal tubules (Ksp-Cre). While impressive, the developing kidney may better provide the full signals required to direct onward renal tubular development than an adult kidney. Yamamoto *et al.* (72) did not direct their murine ES cells in any way. Conventional EB culture also induced the expression of most of the markers that were seen by Kim and Dressler (75). Indeed, these cells went on to form renal structures within the peritoneum of nude mice, suggesting a level of spontaneous renal induction in murine ES cells.

A number of obstacles would remain even once the directed differentiation of hESC toward a renal progenitor fate were achieved. The development of cell isolation techniques will be required to ensure progenitor purity, thereby overcoming the possibility of teratoma formation. Delivery remains an issue, as for other stem cells. In addition, legal barriers and ethical debate about the derivation of hESC remain. Without somatic cell nuclear transfer to generate autologous hESC that are tailored for individual patients, an hESC-based therapy is likely to require immunosuppression, although the data discussed later in this report showing the immunologic protection of embryonic material may mean that immune rejection is less of an issue than expected. Conversely, there is considerable scientific debate over the ability to derive hESC safely using somatic cell nuclear transfer because of our lack of understanding and inability to reprogram genomic imprinting (77). These are obstacles to the adoption of hESC technology in all tissues.

De Novo Bioengineering: A Dream or a Possibility?

Our kidneys filter our entire blood volume 30 times a day, reabsorbing >95% of what is filtered to produce only 1 to 2 L of urine. The kidneys also regulate pH and fluid balance and maintain red blood cell count, BP, and bone density *via* the production of key hormones. The architecture of the kidney is such that the nephrons are aligned with the corresponding tubular sections that are adjacent to each other. This arrangement establishes the countercurrent mechanism that is essential for urinary concentration and, in turn, fluid maintenance and ion balance. These unique spatial constraints and the cellular complexity of this organ make bioengineering a major challenge. A replacement "kidney" can be envisaged as either the complete reengineering of the existing organ or the creation of an alternative structure(s) that is designed to carry out one or more kidney functions.

Bioartificial Glomeruli and Renal Tubules

The kidney was the first organ whose partial function was replaced by an artificial device (78). However, extracorporeal (outside of the body) hemodialysis replaces only the filtration activity of the kidney and not very efficiently. There has been considerable research into the adaptation of this approach *via* the bioengineering of devices to replace filtration or reabsorption (78–83). In the case of filtration, microporous synthetic biocompatible hollow fibers that were coated with MDCK cell extracellular matrix and then seeded with autologous endothelial cells that were harvested from the patient's circulating blood were shown to decrease albumin loss (78,79). For mimicking tubular function, notably resorptive capacity, a renal assist device (RAD) in which renal parenchymal cells are harvested and seeded onto the internal surface of hemodialysis hollow fibers was developed. Blood from the patient is passed along the outside of such fibers. The viability of the seeded cells is maintained *via* oxygen and substrates that are provided by the passing blood and ultrafiltrate (79,84). When tested in animals, these bioartificial tubules provided 40% of normal resorptive capacity. Initially, these two units were used in concert. More recently, the production of a RAD with human cells has been completed successfully and used on humans in an extracorporeal setting (84,85). Here, conventional dialysis was combined with a RAD that contained 10^9 human renal proximal tubular cells that were harvested from donated human kidneys (85). The lack of direct contact between the blood of the patient and these cells allowed a nonautologous cell source to be used. After passing through the hemofilter, patient blood passed through the RAD before being returned to the patient. Ultrafiltrate from the hemofilter also was shunted partially through the RAD to allow reabsorption. This now has been used in phase I/II clinical trials on intensive care unit patients with multiple organ failure, including acute renal failure (85). Such patients normally show a >70% mortality rate even when provided with dialysis. While proving to be safe and apparently improving patient survival, cells within the RAD also demonstrated metabolic and endocrine functions that were appropriate for renal cells and presumably produced chemokines that possibly were critical to patient response. This bioartificial approach using human renal epithelial cells now is referred to by the developing company, RenaMed Biologics, as Renal Bioreplacement Therapy (http://www.nephrostherapeutics.com/) and is being moved into a phase III clinical trial. Could such a unit be implanted for use in chronic renal disease? The challenges to this include the maintenance of patency, reaching a size that is small enough for implantation, and providing the other functions that normally are provided by the kidney. The last may be overcome *via* gene therapy of the cells that are used to seed this apparatus. Seeded cells may even be manipulated to produce their own anticoagulant to assist in maintaining patency. Even if this

challenge is not reached, the use of renal bioreplacement therapy in an extracorporeal setting well may revolutionize the treatment of intensive care unit patients with multiple organ failure.

Recapitulating Development to Create a Kidney De Novo

More than a decade of research already has been devoted to the development of xenotransplantation as an alternative to organ donation (86,87). If all immunologic obstacles could be overcome, then this approach will have a significant impact on the treatment of humans with chronic renal disease. More recently, the use of fetal renal tissue for xenotransplantation has revealed a surprising lack of rejection (88–90). Xenotransplanted embryonic kidney tissue seems to be immunologically protected from the recipient. When transplanted into the abdominal cavity, the embryonic kidney becomes vascularized from the omentum (91,92) and development of functional nephrons proceeds. Such material also can be transplanted successfully into the renal subcapsular space (88,92). The vascularization of such renal primordia is likely to be driven by the expression of vascular endothelial growth factor, which normally acts to draw in developing vasculature from the adjacent aorta during normal kidney formation (93). The immune protection that is afforded such embryonic kidneys seems to exist across concordant (between rodents) or nonconcordant (pig to rodent) barriers. Indeed, anephric rats that were supported by the renal function of a single transplanted metanephros drained by virtue of an ureteroureterostomy have been shown to survive (94). Observations of immune protection of fetal tissue also have been made with liver and pancreas anlage (95). In earlier studies, other groups experienced rejection when transplanting embryonic metanephric slices from one animal to another (96,97). This may be explained by the observation that lack of rejection, subsequent vascularization, and lack of teratoma formation of organ primordia is governed by the collection of that primordia within a defined window of development (98,99). Rogers *et al.* (100) reported that immune protection relates to the absence of donor dendritic cells in early rat anlagen, although immunosuppression is required for rejection to be prevented when crossing more disparate immunologic barriers, such as pig to human (101). Dekel *et al.* (92) investigated the basis of this immune protection *via* expression profiling of adult kidney *versus* fetal grafts. This suggested a reduced expression of a variety of chemokines and proinflammatory factors, suggesting a reduction or immaturity of the innate immune response (90,92).

The knowledge that is gained from metanephric transplantation will be critical for the bioengineering of a *de novo* replacement organ. The advantage of using such fetal material as opposed to stem cells is the inherent organ-specific identity of this tissue, which obviates the need for directed differentiation. Availability also is a significant advantage. However, the possibility of retroviral transmission remains as a question mark over the adoption of such an approach. With the appropriate cells and environment, could nephrogenesis be recapitulated so as to create one or more *de novo* replacement organs in the peritoneal cavity of the patient? Three components would be required: Extracellular matrix, secreted factors, and cells. The potential sources of cells would be the same as for *ex vivo* organ repair with these cells needed to mimic very early MM or even IM. The ability of MSC and undergo nephrogenesis during development was demonstrated by Yokoo *et al.* (60) in whole rodent embryo cultures. Human MSC, engineered to produce GDNF and lacZ using a replication-defective adenoviral construct, were injected into embryonic day 9.5 and 11.5 embryos in the region of the IM that gives rise to the developing kidney. After onward development *ex vivo* for up to 48 h, these cells showed complete contribution to the developing kidney. Understanding how such MSC were directed along a renal developmental program would be needed for their successful use in a *de novo* organ. Yoo *et al.* (102) reported the harvest of adult renal cells that were expanded in culture and seeded onto collagen-coated cylindrical polycarbonate membranes to create an artificial renal tubule. This then was implanted subcutaneously in recipient animals with vascularization occurring from the host. Silicone catheters were connected to these constructs, and after 1 wk, a urine-like filtrate was seen collected in the terminal reservoir. This approach, as anticipated, resulted in an acute rejection. Whatever cell is used, an ability to manipulate these cells genetically also may allow for the regulation of red cell count and bone density. The onward development and neovascularization of transplanted metanephroi can be enhanced *via* the addition of growth hormone, IGF1, hepatocyte growth factor, and fibroblast growth factor 2 (90,103–105). Such research starts to define growth factors that may assist in *de novo* organ generation. The most optimal location for such an organ would be the peritoneal cavity of the patient/recipient with vascularization from the host as for metanephric xenotransplants. Metanephric transplantation vascularized from the ventral body wall mesothelium has been established in the mouse (106). This will enable the use of transgenic mice to examine cell lineage and the relative contribution of recipient to a mini-kidney.

The remaining obstacle to the viability and functionality of a *de novo* peritoneal mini-kidney is the requirement for a ureter plumbed into a bladder. The construction of ureters and bladders is a considerably simpler bioengineering task. There already is a high demand for urinary organ replacements for patients who undergo resections for bladder or ureteric cancer; children who require surgery to repair congenital dysplasia; and patients with urinary incontinence, vesicoureteral reflux, congenital dysplasia, and erectile dysfunction (107). These patients traditionally have had sections of gut used for reconstructions. The use of such mucous-secreting, permeable tissues in an organ such as the bladder creates problems. More recently, silicone or polyglycolic acid–based scaffolds have been used to create replacement parts (107). Replacement bladders have been generated using premolded biodegradable polyglycolic acid fiber matrices onto which urothelial cells and smooth muscle cells have been cultured on the luminal surface (108,109). Replacement bladders that are generated in this way from autologous biopsies now have been implanted successfully in seven patients who required cystectomy for treatment of myelomeningocele (110) (http://www.tengion.com). These patients have been monitored for up to 60 mo and showed

optimal bladder function in those whose implants had the mentum wrapped around them. These patients also displayed normal renal function, no evidence of mucus production, and normal adjacent bowel function (110). A similar approach has been applied to urethras in animal models (111).

Final Hurdles

Although there is excitement about the application of many of these novel regenerative approaches, many hurdles remain, some unique to this organ. These include research obstacles, such as a paucity of assays for clonogenicity and renal potential, which hamper our ability to assess adequately potential renal stem cell populations. The unique architecture of the kidney creates substantial obstacles to the functional integration of a stem cell–derived nephron. Indeed, the functional capacity of a bioengineered organ to provide anything like the filtering and resorptive capacity of the endogenous kidney is doubtful.

The final major obstacle is the degree of damage that is present in a patient with chronic renal disease. It is unlikely than any organ-based repair process will overcome the extent of damage that is seen in a patient who has reached end-stage renal failure. This has major implications for the adoption of any autologous therapy. Even if an adult stem cell population does exist in the adult kidney, would it remain in an end-stage kidney? Indeed, the adoption of any organ-based cellular therapy is likely to succeed only if chronic renal disease can be diagnosed early and if such therapies are implemented well before end-stage renal failure is reached. As we move closer to that point in time, the ethical debate about whether trials can proceed before ESRD will become critical. A lack of surrogate end points with which to assess the success of a cellular therapy in renal disease will make clinical trails long and expensive, eroding the will of the developers to continue to support the trials. However, the imperative to continue to forge such novel approaches is clear from the rate at which the incidence of chronic renal failure is rising in both the developed and the developing world (112–114). In the end, it is unlikely that any such therapies will produce a physiologic outcome that is equivalent to that of a healthy kidney, but as patient numbers inevitably increase the use of dialysis for treatment, a novel therapy that creates an improvement over dialysis will become not only a major achievement but also a necessity.

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References

1. Saxen L: *Organogenesis of the Kidney*, Cambridge, Cambridge University Press, 1987
2. Herzlinger D, Koseki C, Mikawa T, al-Awqati Q: Metanephric mesenchyme contains multipotent stem cells whose fate is restricted after induction. *Development* 114: 565–572, 1992
3. Potter SJ, Thierstein ST: Glomerular development in the kidney as an index of fetal maturity. *J Pediatr* 22: 695–706, 1943
4. Elger M, Hentschel H, Litteral J, Wellner M, Kirsch T, Luft FC, Haller H: Nephrogenesis is induced by partial nephrectomy in the elasmobranch *Leucoraja erinacea*. *J Am Soc Nephrol* 14: 1506–1518, 2003
5. Nagaike M, Hirao S, Tajima H, Noji S, Taniguchi S, Matsumoto K, Nakamura T: Renotropic functions of hepatocyte growth factor in renal regeneration after unilateral nephrectomy. *J Biol Chem* 266: 22781–22784, 1991
6. Cochrane AL, Kett MM, Samuel CS, Campanale NV, Anderson WP, Hume DA, Little MH, Bertram JF, Ricardo SD: Renal structural and functional repair in a mouse model of reversal of ureteral obstruction. *J Am Soc Nephrol* 16: 3623–3630, 2005
7. Strutz F, Okada H, Lo CW, Danoff T, Carone RL, Tomaszewski JE, Neilson EG: Identification and characterization of a fibroblast marker: FSP1. *J Cell Biol* 130: 393–405, 1995
8. Witzgall R, Brown D, Schwarz C, Bonventre JV: Localization of proliferating cell nuclear antigen, vimentin, c-Fos, and clusterin in the postischemic kidney. Evidence for a heterogenous genetic response among nephron segments, and a large pool of mitotically active and dedifferentiated cells. *J Clin Invest* 93: 2175–2188, 1994
9. Bonventre JV: Dedifferentiation and proliferation of surviving epithelial cells in acute renal failure. *J Am Soc Nephrol* 14[Suppl 1]: S55–S61, 2003
10. Cornacchia F, Fornoni A, Plati AR, Thomas A, Wang Y, Inverardi L, Striker LJ, Striker GE: Glomerulosclerosis is transmitted by bone marrow-derived mesangial cell progenitors. *J Clin Invest* 108: 1649–1656, 2001
11. Imasawa T, Utsunomiya Y, Kawamura T, Zhong Y, Nagasawa R, Okabe M, Maruyama N, Hosoya T, Ohno T: The potential of bone marrow-derived cells to differentiate to glomerular mesangial cells. *J Am Soc Nephrol* 12: 1401–1409, 2001
12. Poulsom R, Forbes SJ, Hodivala-Dilke K, Ryan E, Wyles S, Navaratnarasah S, Jeffery R, Hunt T, Alison M, Cook T, Pusey C, Wright NA: Bone marrow contributes to renal parenchymal turnover and regeneration. *J Pathol* 195: 229–235, 2001
13. Ito T, Suzuki A, Imai E, Okabe M, Hori M: Bone marrow is a reservoir of repopulating mesangial cells during glomerular remodeling. *J Am Soc Nephrol* 12: 2625–2635, 2001
14. Ito T, Suzuki A, Okabe M, Imai E, Hori M: Application of bone marrow-derived stem cells in experimental nephrology. *Exp Nephrol* 9: 444–450, 2001
15. Ricardo SD, Deane, JA: Adult stem cells in renal injury and repair. *Nephrology (Carlton)* 10: 276–282, 2005
16. Douglas-Denton R, Moritz KM, Bertram JF, Wintour EM: Compensatory renal growth after unilateral nephrectomy in the ovine fetus. *J Am Soc Nephrol* 13: 406–410, 2002
17. Hayakawa M, Ishizaki M, Hayakawa J, Migita M, Murakami M, Shimada T, Fukunaga Y: Role of bone marrow cells in the healing process of mouse experimental glomerulonephritis. *Pediatr Res* 58: 323–328, 2005
18. Ikarashi K, Li B, Suwa M, Kawamura K, Morioka T, Yao J, Khan F, Uchiyama M, Oite T: Bone marrow cells contribute

to regeneration of damaged glomerular endothelial cells. *Kidney Int* 67: 1925–1933, 2005
19. Camargo FD, Chambers SM, Goodell MA: Stem cell plasticity: From transdifferentiation to macrophage fusion. *Cell Prolif* 37: 55–65, 2004
20. Camargo FD, Finegold M, Goodell MA: Hematopoietic myelomonocytic cells are the major source of hepatocyte fusion partners. *J Clin Invest* 113: 1266–1270, 2004
21. Weimann JM, Johansson CB, Trejo A, Blau HM: Stable reprogrammed heterokaryons form spontaneously in Purkinje neurons after bone marrow transplant. *Nat Cell Biol* 5: 959–966, 2003
22. Lin F, Moran A, Igarashi P: Intrarenal cells, not bone marrow-derived cells, are the major source for regeneration in postischemic kidney. *J Clin Invest* 115: 1756–1764, 2005
23. Duffield JS, Park KM, Hsiao LL, Kelley VR, Scadden DT, Ichimura T, Bonventre JV: Restoration of tubular epithelial cells during repair of the postischemic kidney occurs independently of bone marrow-derived stem cells. *J Clin Invest* 115: 1743–1755, 2005
24. Fang TC, Alison MR, Cook HT, Jeffery R, Wright NA, Poulsom R: Proliferation of bone marrow-derived cells contributes to regeneration after folic acid-induced acute tubular injury. *J Am Soc Nephrol* 16: 1723–1732, 2005
25. Nishida M, Okumura Y, Fujimoto S, Shiraishi I, Itoi T, Hamaoka K: Adoptive transfer of macrophages ameliorates renal fibrosis in mice. *Biochem Biophys Res Commun* 332: 11–16, 2005
26. Imasawa T, Nagasawa R, Utsunomiya Y, Kawamura T, Zhong Y, Makita N, Muso E, Miyawaki S, Maruyama N, Hosoya T, Sakai O, Ohno T: Bone marrow transplantation attenuates murine IgA nephropathy: Role of a stem cell disorder. *Kidney Int* 56: 1809–1817, 1999
27. Stokman G, Leemans JC, Claessen N, Weening JJ, Florquin S: Hematopoietic stem cell mobilization therapy accelerates recovery of renal function independent of stem cell contribution. *J Am Soc Nephrol* 16: 1684–1692, 2005
28. Iwasaki M, Adachi Y, Minamino K, Suzuki Y, Zhang Y, Okigaki M, Nakano K, Koike Y, Wang J, Mukaide H, Taketani S, Mori Y, Takahashi H, Iwasaka T, Ikehara S: Mobilization of bone marrow cells by G-CSF rescues mice from cisplatin-induced renal failure, and M-CSF enhances the effects of G-CSF. *J Am Soc Nephrol* 16: 658–666, 2005
29. Held PK, Al-Dhalimy M, Willenbring H, Akkari Y, Jiang S, Torimaru Y, Olson S, Fleming WH, Finegold M, Grompe, M: In vivo genetic selection of renal proximal tubules. *Mol Ther* 13: 49–58, 2006
30. Sugimoto H, Mundel TM, Sund M, Xie L, Cosgrove D, Kalluri R: Bone-marrow-derived stem cells repair basement membrane collagen defects and reverse genetic kidney disease. *Proc Natl Acad Sci U S A* 103: 7321–7326, 2006
31. Brockes JP, Kumar A: Plasticity and reprogramming of differentiated cells in amphibian regeneration. *Nat Rev Mol Cell Biol* 3: 566–574, 2002
32. Brockes JP: Amphibian limb regeneration: Rebuilding a complex structure. *Science* 276: 81–87, 1997
33. Brockes JP, Kumar A: Appendage regeneration in adult vertebrates and implications for regenerative medicine. *Science* 310: 1919–1923, 2005
34. Kondo T, Raff M: Oligodendrocyte precursor cells reprogrammed to become multipotential CNS stem cells. *Science* 289: 1754–1757, 2000
35. Odelberg SJ, Kollhoff A, Keating MT: Dedifferentiation of mammalian myotubes induced by msx1. *Cell* 103: 1099–1109, 2000
36. Leferovich JM, Bedelbaeva K, Samulewicz S, Zhang XM, Zwas D, Lankford EB, Heber-Katz E: Heart regeneration in adult MRL mice. *Proc Natl Acad Sci U S A* 98: 9830–9835, 2001
37. Azizi SA: Exploiting nonneural cells to rebuild the nervous system: From bone marrow to brain. *Neuroscientist* 5: 353–361, 2000
38. Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR: Turning blood into brain: Cells bearing neuronal antigens generated in vivo from bone marrow. *Science* 290: 1779–1782, 2000
39. Rietze RL, Valcanis H, Brooker GF, Thomas T, Voss AK, Bartlett PF: Purification of a pluripotent neural stem cell from the adult mouse brain. *Nature* 412: 736–739, 2001
40. Toma JG, Akhavan M, Fernandes KJ, Barnabe-Heider F, Sadikot A, Kaplan DR, Miller FD: Isolation of multipotent adult stem cells from the dermis of mammalian skin. *Nat Cell Biol* 3: 778–784, 2001
41. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH: Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Eng* 7: 211–228, 2001
42. Burrow CR, Wilson PD: A putative Wilms tumor-secreted growth factor activity required for primary culture of human nephroblasts. *Proc Natl Acad Sci U S A* 90: 6066–6070, 1993
43. Kitamura S, Yamasaki Y, Kinomura M, Sugaya T, Sugiyama H, Maeshima Y, Makino H: Establishment and characterization of renal progenitor like cells from S3 segment of nephron in rat adult kidney. *FASEB J* 19: 1789–1797, 2005
44. Challen GA, Martinez G, Davis MJ, Taylor DF, Crowe M, Teasdale RD, Grimmond SM, Little MH: Identifying the molecular phenotype of renal progenitor cells. *J Am Soc Nephrol* 15: 2344–2357, 2004
45. Miraglia S, Godfrey W, Yin AH, Atkins K, Warnke R, Holden JT, Bray RA, Waller EK, Buck DW: A novel five-transmembrane hematopoietic stem cell antigen: Isolation, characterization, and molecular cloning. *Blood* 90: 5013–5021, 1997
46. Bussolati B, Bruno S, Grange C, Buttiglieri S, Deregibus MC, Cantino D, Camussi G: Isolation of renal progenitor cells from adult human kidney. *Am J Pathol* 166: 545–555, 2005
47. Maeshima A, Yamashita S, Nojima Y: Identification of renal progenitor-like tubular cells that participate in the regeneration processes of the kidney. *J Am Soc Nephrol* 14: 3138–3146, 2003
48. Yamashita S, Maeshima A, Nojima Y: Involvement of renal progenitor tubular cells in epithelial-to-mesenchymal transition in fibrotic rat kidneys. *J Am Soc Nephrol* 16: 2044–2051, 2005
49. Maeshima A, Sakurai H, Nigam SK: Adult kidney tubular cell population showing phenotypic plasticity, tubulogenic capacity, and integration capability into developing kidney. *J Am Soc Nephrol* 17: 188–198, 2006
50. Vogetseder A, Karadeniz A, Kaissling B, Le Hir M: Tubular cell proliferation in the healthy rat kidney. *Histochem Cell Biol* 124: 97–104, 2005

51. Oliver JA, Maarouf O, Cheema FH, Martens TP, Al-Awqati Q: The renal papilla is a niche for adult kidney stem cells. *J Clin Invest* 114: 795–804, 2004
52. Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC: Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. *J Exp Med* 183: 1797–1806, 1996
53. Asakura A, Rudnicki MA: Side population cells from diverse adult tissues are capable of in vitro hematopoietic differentiation. *Exp Hematol* 30: 1339–1345, 2002
54. Hishikawa K, Marumo T, Miura S, Nakanishi A, Matsuzaki Y, Shibata K, Ichiyanagi T, Kohike H, Komori T, Takahashi I, Takase O, Imai N, Yoshikawa M, Inowa T, Hayashi M, Nakaki T, Nakauchi H, Okano H, Fujita T: Musculin/MyoR is expressed in kidney side population cells and can regulate their function. *J Cell Biol* 169: 921–928, 2005
55. Iwatani H, Ito T, Imai E, Matsuzaki Y, Suzuki A, Yamato M, Okabe M, Hori M: Hematopoietic and nonhematopoietic potentials of Hoechst(low)/side population cells isolated from adult rat kidney. *Kidney Int* 65: 1604–1614, 2004
56. Hishikawa K: Do we really need "stem" cell for renal regeneration? *Kidney Int* 68: 1966–1967, 2005
57. Challen GA, Bertoncello I, Deane JA, Ricardo SD, Little MH: Kidney side population reveals multilineage potential and renal functional capacity but also cellular heterogeneity. *J Am Soc Nephrol* 17: 1896–1912, 2006
58. Challen GA, Little MH: A side order of stem cells: The SP phenotype. *Stem Cells* 24: 3–12, 2006
59. Horwitz EM, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC, Deans RJ, Krause DS, Keating A: Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy* 7: 393–395, 2005
60. Yokoo T, Ohashi T, Shen JS, Sakurai K, Miyazaki Y, Utsunomiya Y, Takahashi M, Terada Y, Eto Y, Kawamura T, Osumi N, Hosoya T: Human mesenchymal stem cells in rodent whole-embryo culture are reprogrammed to contribute to kidney tissues. *Proc Natl Acad Sci U S A* 102: 3296–3300, 2005
61. Herrera MB, Bussolati B, Bruno S, Fonsato V, Romanazzi GM, Camussi G: Mesenchymal stem cells contribute to the renal repair of acute tubular epithelial injury. *Int J Mol Med* 14: 1035–1041, 2004
62. Morigi M, Imberti B, Zoja C, Corna D, Tomasoni S, Abbate M, Rottoli D, Angioletti S, Benigni A, Perico N, Alison M, Remuzzi G: Mesenchymal stem cells are renotropic, helping to repair the kidney and improve function in acute renal failure. *J Am Soc Nephrol* 15: 1794–1804, 2004
63. Lange C, Togel F, Ittrich H, Clayton F, Nolte-Ernsting C, Zander AR, Westenfelder C: Administered mesenchymal stem cells enhance recovery from ischemia/reperfusion-induced acute renal failure in rats. *Kidney Int* 68: 1613–1617, 2005
64. Togel F, Hu Z, Weiss K, Isaac J, Lange C, Westenfelder C: Administered mesenchymal stem cells protect against ischemic acute renal failure through differentiation-independent mechanisms. *Am J Physiol Renal Physiol* 289: F31–F42, 2005
65. Luttichaux IV, Notohamiprodjo M, Wechselberger A, Peters C, Henger A, Seliger C, Djafarzadeh R, Huss R, Nelson PJ: Human adult CD34-progenitor cells functionally express the chemokine receptors CCR1, CCR4, CCR7, CXCR5, and CCR10 but not CXCR4. *Stem Cells Dev* 14: 329–336, 2005
66. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM: Embryonic stem cell lines derived from human blastocysts. *Science* 282: 1145–1147, 1998
67. Reubinoff BE, Pera MF, Fong CY, Trounson A, Bongso A: Embryonic stem cell lines from human blastocysts: Somatic differentiation in vitro. *Nat Biotechnol* 18: 399–404, 2000
68. Shamblott MJ, Axelman J, Wang S, Bugg EM, Littlefield JW, Donovan PJ, Blumenthal PD, Huggins GR, Gearhart JD: Derivation of pluripotent stem cells from cultured human primordial germ cells. *Proc Natl Acad Sci U S A* 95: 13726–13731, 1998
69. Pera MF: Human pluripotent stem cells: A progress report. *Curr Opin Genet Dev* 11: 595–599, 2001
70. Kouskoff V, Lacaud G, Schwantz S, Fehling HJ, Keller G: Sequential development of hematopoietic and cardiac mesoderm during embryonic stem cell differentiation. *Proc Natl Acad Sci U S A* 102: 13170–13175, 2005
71. Singla DK, Sobel BE: Enhancement by growth factors of cardiac myocyte differentiation from embryonic stem cells: A promising foundation for cardiac regeneration. *Biochem Biophys Res Commun* 335: 637–642, 2005
72. Yamamoto M, Cui L, Johkura K, Asanuma K, Okouchi Y, Ogiwara N, Sasaki K: Branching ducts similar to mesonephric ducts or ureteric buds in teratomas originating from mouse embryonic stem cells. *Am J Physiol Renal Physiol* 290: F52–F60, 2006
73. Steenhard BM, Isom KS, Cazcarro P, Dunmore JH, Godwin AR, St John PL, Abrahamson DR: Integration of embryonic stem cells in metanephric kidney organ culture. *J Am Soc Nephrol* 16: 1623–1631, 2005
74. Kobayashi T, Tanaka H, Kuwana H, Inoshita S, Teraoka H, Sasaki S, Terada Y: Wnt4-transformed mouse embryonic stem cells differentiate into renal tubular cells. *Biochem Biophys Res Commun* 336: 585–595, 2005
75. Kim D, Dressler GR: Nephrogenic factors promote differentiation of mouse embryonic stem cells into renal epithelia. *J Am Soc Nephrol* 16: 3527–3534, 2005
76. Osafune K, Nishinakamura R, Komazaki S, Asashima M: In vitro induction of the pronephric duct in *Xenopus* explants. *Dev Growth Differ* 44: 161–167, 2002
77. Jaenisch R: Human cloning: The science and ethics of nuclear transplantation. *N Engl J Med* 351: 2787–2791, 2004
78. Humes HD: Bioartificial kidney for full renal replacement therapy. *Semin Nephrol* 20: 71–82, 2000
79. Humes HD, MacKay SM, Funke AJ, Buffington DA: Tissue engineering of a bioartificial renal tubule assist device: In vitro transport and metabolic characteristics. *Kidney Int* 55: 2502–2514, 1999
80. Yoo JJ, Atala A: Tissue engineering applications in the genitourinary tract system. *Yonsei Med J* 41: 789–802, 2000
81. Amiel GE, Yoo JJ, Atala A: Renal therapy using tissue-engineered constructs and gene delivery. *World J Urol* 18: 71–79, 2000
82. Koh CJ, Atala A: Therapeutic cloning and tissue engineering. *Curr Top Dev Biol* 60: 1–15, 2004
83. Tiranathanagul K, Eiam-Ong S, Humes HD: The future of renal support: High-flux dialysis to bioartificial kidneys. *Crit Care Clin* 21: 379–394, 2005

84. Humes HD, Fissell WH, Weitzel WF, Buffington DA, Westover AJ, MacKay SM, Gutierrez JM: Metabolic replacement of kidney function in uremic animals with a bioartificial kidney containing human cells. *Am J Kidney Dis* 39: 1078–1087, 2002
85. Humes HD, Weitzel WF, Bartlett RH, Swaniker FC, Paganini EP, Luderer JR, Sobota J: Initial clinical results of the bioartificial kidney containing human cells in ICU patients with acute renal failure. *Kidney Int* 66: 1578–1588, 2004
86. Dwyer KM, Cowan PJ, d'Apice AJ: Xenotransplantation: Past achievements and future promise. *Heart Lung Circ* 11: 32–41, 2002
87. Toledo-Pereyra LH, Lopez-Neblina F: Xenotransplantation: A view to the past and an unrealized promise to the future. *Exp Clin Transplant* 1: 1–7, 2003
88. Rogers NJ, Dorling A, Moore M: Xenotransplantation: Steps towards a clinical reality. *Immunol Today* 19: 206–208, 1998
89. Hammerman MR: Organogenesis of kidneys following transplantation of renal progenitor cells. *Transpl Immunol* 12: 229–239, 2004
90. Dekel B, Amariglio N, Kaminski N, Schwartz A, Goshen E, Arditti FD, Tsarfaty I, Passwell JH, Reisner Y, Rechavi G: Engraftment and differentiation of human metanephroi into functional mature nephrons after transplantation into mice is accompanied by a profile of gene expression similar to normal human kidney development. *J Am Soc Nephrol* 13: 977–990, 2002
91. Hammerman MR: Transplantation of embryonic kidneys. *Clin Sci (Lond)* 103: 599–612, 2002
92. Dekel B, Burakova T, Arditti FD, Reich-Zeliger S, Milstein O, Aviel-Ronen S, Rechavi G, Friedman N, Kaminski N, Passwell JH, Reisner Y: Human and porcine early kidney precursors as a new source for transplantation. *Nat Med* 9: 53–60, 2003
93. Akimoto T, Hammerman MR: Microvessel formation from mouse aorta is stimulated in vitro by secreted VEGF and extracts from metanephroi. *Am J Physiol Cell Physiol* 284: C1625–C1632, 2003
94. Rogers SA, Hammerman MR: Prolongation of life in anephric rats following de novo renal organogenesis. *Organogenesis* 1: 22–25, 2004
95. Rogers SA, Chen F, Talcott M, Hammerman MR: Islet cell engraftment and control of diabetes in rats after transplantation of pig pancreatic anlagen. *Am J Physiol Endocrinol Metab* 286: E502–E509, 2004
96. Woolf AS, Loughna S: Origin of glomerular capillaries: Is the verdict in? *Exp Nephrol* 6: 17–21, 1998
97. Abrahamson DR, St John PL, Pillion DJ, Tucker DC: Glomerular development in intraocular and intrarenal grafts of fetal kidneys. *Lab Invest* 64: 629–639, 1991
98. Eventov-Friedman S, Katchman H, Shezen E, Aronovich A, Tchorsh D, Dekel B, Freud E, Reisner Y: Embryonic pig liver, pancreas, and lung as a source for transplantation: Optimal organogenesis without teratoma depends on distinct time windows. *Proc Natl Acad Sci U S A* 102: 2928–2933, 2005
99. Hammerman MR: Windows of opportunity for organogenesis. *Transpl Immunol* 15: 1–8, 2005
100. Rogers SA, Hammerman MR: Transplantation of rat metanephroi into mice. *Am J Physiol Regul Integr Comp Physiol* 280: R1865–R1869, 2001
101. Rogers SA, Talcott M, Hammerman MR: Transplantation of pig metanephroi. *ASAIO J* 49: 48–52, 2003
102. Yoo JJ, Ashkar S, Atala A: Creation of functional kidney structures with excretion of urine-like fluid in vivo. *Pediatrics* 98: 605, 1996
103. Rogers SA, Powell-Braxton L, Hammerman MR: Insulin-like growth factor I regulates renal development in rodents. *Dev Genet* 24: 293–298, 1999
104. Hammerman MR: The growth hormone-insulin-like growth factor axis in kidney re-revisited. *Nephrol Dial Transplant* 14: 1853–1860, 1999
105. Akimoto T, Hammerman MR: Fibroblast growth factor 2 promotes microvessel formation from mouse embryonic aorta. *Am J Physiol Cell Physiol* 284: C371–C377, 2003
106. Armstrong SR, Campbell GR, Campbell JH, Little MH: Establishment of metanephros transplantation in mice highlights contributions by both nephrectomy and pregnancy to developmental progression. *Nephron Exp Nephrol* 101: e155–e164, 2005
107. Park KD, Kwon IK, Kim YH: Tissue engineering of urinary organs. *Yonsei Med J* 41: 780–788, 2000
108. Atala A, Vacanti JP, Peters CA, Mandell J, Retik AB, Freeman MR: Formation of urothelial structures in vivo from dissociated cells attached to biodegradable polymer scaffolds in vitro. *J Urol* 148: 658–662, 1992
109. Oberpenning F, Meng J, Yoo JJ, Atala A: De novo reconstitution of a functional mammalian urinary bladder by tissue engineering. *Nat Biotechnol* 17: 149–155, 1999
110. Atala A, Bauer SB, Soker S, Yoo JJ, Retik AB: Tissue-engineered autologous bladders for patients needing cystoplasty. *Lancet* 367: 1241–1246, 2006
111. Cilento BG, Retik AB, Atala A: Urethral reconstruction using a polymer scaffolds seeded with urothelial and smooth muscle cells. *J Urol* 155: 5, 1996
112. Xue JL, Ma JZ, Louis TA, Collins AJ: Forecast of the number of patients with end-stage renal disease in the United States to the year 2010. *J Am Soc Nephrol* 12: 2753–2758, 2001
113. Correa-Rotter R, Naicker S, Katz IJ, Agarwal SK, Herrera Valdes R, Kaseje D, Rodriguez-Iturbe B, Shaheen F, Sitthi-Amorn C: Demographic and epidemiologic transition in the developing world: Role of albuminuria in the early diagnosis and prevention of renal and cardiovascular disease. *Kidney Int Suppl* 92: S32–S37, 2004
114. Alebiosu CO, Ayodele OE: The global burden of chronic kidney disease and the way forward. *Ethn Dis* 15: 418–423, 2005