

Stem cells in the kidney

QAIS AL-AWQATI and JUAN A. OLIVER

Departments of Medicine and Physiology and Cellular Biophysics, College of Physicians & Surgeons of Columbia University, New York New York, USA

Stem cells in the kidney. The kidney is derived from the ureteric bud and the metanephrogenic mesenchyme, and these two progenitor cells differentiate into more than 26 different cell types in the adult kidney. The ureteric bud contains the precursor of the epithelial cells of the collecting duct and the renal mesenchyme contains precursors of all the epithelia of the rest of the nephron, endothelial cell precursors and stroma cells, but the relatedness among these cells is unclear. A single metanephric mesenchymal cell can generate all the epithelial cells of the nephron (except the collecting duct), indicating that the kidney contains epithelial stem cells. It is currently unknown whether these stem cells also are present in the adult kidney but experience in other organs makes this likely. It also is unclear whether embryonic renal epithelial stem cells can generate other cell types, but preliminary studies in our laboratory suggest that they can differentiate into myofibroblasts, smooth muscle, and perhaps endothelial cells, indicating that they are pluripotent renal stem cells. The important problem to be solved now is the identification and location of adult renal stem cells. This article discusses work done in other organs and in renal development that we believe may be useful for the resolution of this problem.

When the World Wide Web is searched for the key word stem cells, more than 450,000 sites are found! This tremendous public interest in a subject that is fundamentally a purely scientific issue, albeit with great potential applications, is a welcome development in the dissemination of scientific knowledge. The potential of stem cell therapy even has created a new field of research: Regenerative Medicine [1]. Stem cells, especially those of hemopoiesis are already being used for repopulation of the bone marrow after near lethal chemotherapy. There is no doubt that in the foreseeable future stem cell therapy will acquire a central role in the treatment of diseases of most organs where they will be used to repopulate organs damaged by disease, to “reconstitute” organs in

vitro for transplantation or to deliver genes to privileged sites. Because there is very little that is known regarding stem cells in the kidney, this review will emphasize what has been achieved in other fields and outlines the context where further research in the kidney could bring us to an understanding of the origin of renal cells and answer the question of whether the adult kidney possesses organ specific stem cells.

EMBRYONIC STEM CELLS

After several divisions of the fertilized egg, the cells begin to adhere to each other in the morula and the surface cells of this sphere begin to differentiate and form the epithelium of the trophectoderm, the first differentiated structure in the embryo. The apical side of this epithelium faces outward and the cells begin to transport NaCl and water into the inner cavity of this blastocyst. While this epithelium is critical for implantation and the formation of the placenta, it is the inner cell mass that will form the embryo. The cells of this mass are the embryonic stem (ES) cells and will differentiate into ectoderm, mesoderm and endoderm (Fig. 1). ES cells can form any tissue in the embryo and their use has revolutionized mouse developmental biology. Introduction of ES cells whose genetic complement has been altered by transfection or deletion of genes into mouse blastocysts allows the generation of animals that carry the transgenes or the mutations. Incidentally, ES cells are very difficult to culture and propagate, and in only one strain of mice (SvJ 129) have the conditioned cells been sufficiently defined to make the work routinely feasible.

While embryonic stem cells are “totipotent” and can generate any cell in any organ, it is likely that during embryonic development (and during adult life) different organs possess organ-specific stem cells that derive from ES cells and can give rise to most if not all the cells present in an organ. Hence, one could state that ES cells could generate organ-specific stem cells that would then produce lineage-restricted cell types. This somewhat idealized situation had to be revised recently when it was discovered that at least some organ-specific stem cells had

Key words: uteric bud, metanephron, renal mesenchyme, growth and development, embryonic stem cells, adult stem cells, kidney development.

Received for publication July 30, 2001
and in revised form October 2, 2001
Accepted for publication October 23, 2001

© 2002 by the International Society of Nephrology

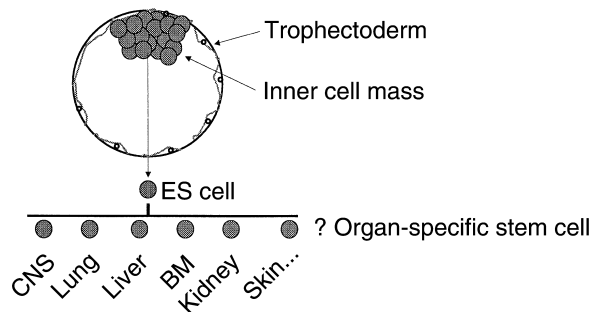


Fig. 1. Components of the blastocyst and development of organ-specific stem cells.

the unsuspected plasticity of being capable of producing many different lineages. For instance, studies clearly demonstrate that the progeny of a single hemopoietic stem cell (HSC) injected into irradiated mice can generate epithelial cells of the intestine, lung and liver, as well as, of course, blood cells.

CHARACTERISTICS OF ORGAN-SPECIFIC STEM CELLS

Multipotency

The *sine qua non* of stem cells is that they are able to generate many or all the differentiated cell types in an organ. For instance, a single hemopoietic stem cell can repopulate the bone marrow, spleen and blood of lethally irradiated mice. A single neural stem cell taken from the ependyma of the brain can generate astrocytes, glia and neurons [2]. The emphasis on studies where a single stem cell is followed is intentional since it is easy to define stem cells, but difficult to identify them. Many ambiguities have resulted from injection of poorly characterized groups of cells, only some of which were likely to be true stem cells.

Self-generation and asymmetric division

Multipotency has been supplemented by another criterion, that a stem cell upon mitosis can regenerate itself [3]. Hence, the product of a cell division of a stem cell is asymmetric in that it generates a differentiated cell and regenerates itself. However, while asymmetric cell division clearly occurs at the single cell level, some stem cell divisions are asymmetric only when a whole population of daughter cells is examined. Division of a single stem cell to produce two daughter cells that have different proteins or mRNA implies that asymmetry existed before cell division, a finding recently documented in *Drosophila* and *C. elegans* [4]. For instance, Numb, a membrane protein that is critical for specification of neural cell identity, is localized asymmetrically during *Drosophila* neuroblast mitosis [4]. While diffusely distributed initially,

Numb forms a basal crescent during prophase that persists into later stages of the cell cycle. After division, Numb is preferentially segregated into the basal surface of only one of the daughter cells, the ganglion mother cell (GMC).

Cycling time

Other characteristics of stem cells have been developed recently that reflect their behavior during conversion into more restricted phenotypes. It was found that the turnover time of stem cells in bone marrow and skin was very slow while their progeny divided vigorously at a rapid rate [5]. Only 10% of hemopoietic stem cells enter the cell cycle per day and all of them do so within one to three months, a very slow rate indeed. This has formed the basis of a useful assay, the retention of a nucleotide label named bromo-deoxyuridine (BrdU). Cells are initially labeled and then the BrdU is washed off and cells observed for long periods of "chase." When a cell divides rapidly in the absence of the label, the BrdU content of the daughter cells becomes dilute. Only the slow cycling cells will retain a high enough concentration of BrdU to allow its detection, and hence, stem cells are often termed Label-retaining cells. Their immediate progeny divide rapidly and hence will have a sufficiently dilute concentration to escape detection. These cells are termed Transit Amplifying Cells (Fig. 2). Eventually, these cells will terminally differentiate and their cycling rate is usually slow, but it varies in different organs [6].

Stem cells in vitro

When stem cells are isolated and grown in culture, they often undergo rapid multiplication, at least initially. Often these divisions are not asymmetric, that is, they are regenerative rather than differentiative. In many cases maintenance of the "stemness" requires special growth factors. An important aim of stem cell research is to identify the factors that allow the expansion of stem cells in culture with the aim of producing enough for therapeutic purposes. Basic fibroblast growth factor (bFGF) was found to be necessary for expansion of neural stem cells and of hemopoietic stem cells in culture [7]. Recently, Barasch et al found it to be necessary for survival of the metanephric mesenchyme to allow its conversion to epithelia [8]. Withdrawal of this factor in the case of neural stem cells leads to quiescence and to the ability to undergo asymmetric division and generation of differentiated progeny.

Location in a niche

Stem cells are often located in a specific niche with ready access to an abundant blood supply and where they are protected from obvious sources of environmental damage [9]. It is likely that these niches allow interaction between these stem cells and other cells that might pro-

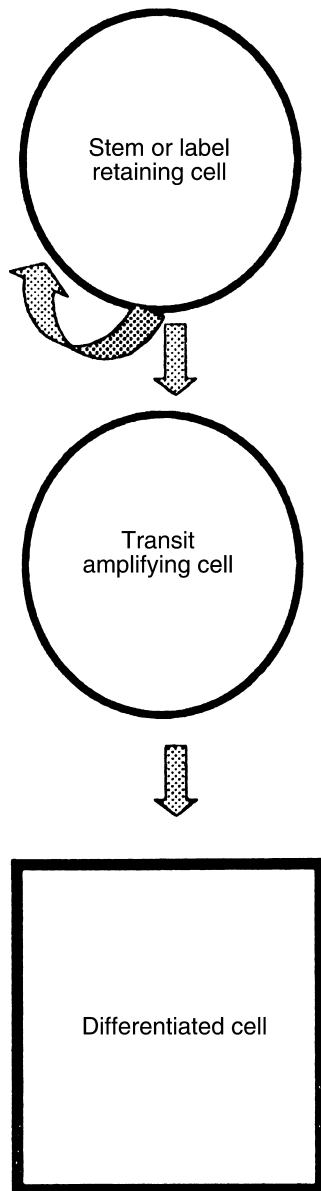


Fig. 2. Pathway of differentiation of stem cells.

duce factors that prevent rapid cycling. For instance, it is well known that in vitro culture of stem cells results in a burst of accelerated cell division, suggesting the presence of factors in the niche that prevent rapid cycling. Other factors present in these niches might support and promote all the characteristics that make a stem cell achieve asymmetric division or slow cycling. Further, several of these niches are located in privileged regions that protect against environmental damage. For instance, stem cells of the skin are located in the hair follicle, deep in the dermis often surrounded by pigment cells to protect them from UV light. Similarly, the corneal stem cells are located in the limbus in a region that is well vascularized and surrounded by pigmented cells. In addition,

the extremely slow capacity of the stem cell to divide protects the cell from DNA damage.

Are stem cells undifferentiated?

Because stem cells generate differentiated cells, there is a tendency to think of them as undifferentiated. Nothing could be further from the truth. Stem cells have a specific complement of genes that are only expressed in them. Indeed, one of the major aims of stem cell research is to characterize all the unique proteins that are only expressed in them. So far, only a few of these proteins have been isolated in the case of hemopoietic stem cells; they have been used to isolate these cells from mouse bone marrow using fluorescence-activated cell sorter (FACS) analysis, and mouse stem cells are currently defined as $AA4^+SCA^+Kit^+Lin^{-\text{ or low}}Thy1.1^{-\text{ or low}}$ [1, 2, 9]. In human cells, CD34 is traditionally considered to be unique for bone marrow stem cells, although it now seems that when CD34⁺ cells are allowed to reside and localize in the bone marrow, they down-regulate this protein. Keratinocyte stem cells express high levels of α_6 integrin and low levels of the transferrin receptor. Single neural stem cells with capacity to generate neurons and glia express CD133, but not CD34 or CD45. Repeated isolation from the initial FACS-sorted population showed that these cells maintained this expression pattern [10]. Recent studies using microarray gene chips have demonstrated that hemopoietic stem cells have a specific complement of expressed genes that are not found in their progeny [11]. Many of these genes are novel and have no known homologs in the database. About a fourth are membrane proteins demonstrating the critical importance of interaction with the environment. Remarkably, a few genes are present in both hemopoietic and neural stem cells, suggesting that these genes might underlie some of the fundamental characteristics described above [11]. There is an urgent need to characterize the proteins that are expressed only in stem cells. Without this knowledge neither isolation nor study of these cells can proceed with any degree of efficiency.

Stem cells in adult organs

The idea that stem cells must be present in the adult animal came about when it was demonstrated that the turnover of differentiated cells in some organs was fast. To replenish these cells, some multipotent progenitor had to be present in the bone marrow, skin, and the small intestine. Currently there is a general belief that all adult organs contain stem cells, but that we have not developed tools to recognize them either by location or by characteristic morphology or surface molecule expression. The question of whether stem cells located in adult tissues are the same as the ones that are present in embryonic development is yet to be answered. Recent studies of hemopoietic stem cells isolated from adult

bone marrow or fetal liver have shown surprising differences in gene expression [11]. The fetal liver stem cells have a broader developmental potential and seem to have a higher rate of cell cycle entry than the adult bone marrow-derived cells.

The new alchemy or can blood turn into brain

There seems to be no end to reports in scientific and popular media that purport to show that stem cells taken from one organ, especially from an adult animal, can differentiate into other organs. For instance, transplantation of neural stem cells grown in clonal cultures into sub-lethally irradiated mice led to the appearance hemopoietic cells [12]. Transplantation of bone marrow or some blood cells that share at least some markers with hemopoietic stem cells have resulted in the donor progeny populating endothelial cells [13] and skeletal muscle [14]. Bone marrow cells also have been implicated in hepatic regeneration [15] in the formation of central nervous system (CNS) cell types [16] and the generation of myocardial cells [17]. Infusion of muscle stem cells (satellite stem cells) also has led to the generation of bone marrow-derived cells [18, 19].

If true, the ability of these stem cells to “transdifferentiate” could revolutionize therapy, since it will allow a large choice of starting material as well as development of protocols to help expand these cells *in vitro*. Incidentally, it also would eliminate the need for embryonic stem cells with their lethal political baggage. However, it has been pointed out that several criteria need to be satisfied before these findings can be accepted [20]. The idea that organ-specific stem cells are “lineage-restricted,” that is, their progeny can differentiate along only a specific path, is one of the classical dicta of embryology. A large amount of research based on transplantation of individual or several cells from one region of the embryo to another shows that these cells lose the ability to be “re-specified” by their new environment. So what is the explanation of these new findings? Could it be that what is being isolated is really an earlier stage of cellular development, that is, that bone marrow might contain itinerant ES cells? Because this is pretty unlikely, we emphasize the need for a quantitative analysis of the progeny of such transdifferentiating cells. In one of the most convincing studies, a single labeled hemopoietic stem cell taken from one mouse was injected into another and it homed to the bone marrow. These labeled cells were then extracted from the bone marrow and injected into lethally irradiated mice [21]. The progeny of this cell populated lung, intestine liver and skin as well as the expected bone marrow-derived blood cells. However, what fraction of the progeny of this single stem cell produced these different epithelial cells compared to the blood cells is unclear. If these epithelial cells were rare, then one has to consider the possibility that the fate of the progeny is largely

pre-determined to form blood cells but there is an occasional stochastic fluctuation. On the one hand, these studies do suggest that it is possible for an organ-specific stem cell to transdifferentiate. On the other hand, unless this transdifferentiation occurs with great frequency, they will not be of much use in re-populating or replacing diseased organs.

If transdifferentiation turns out to be a general property, it suggests that organ-specific stem cells regardless of their habitat or origin must share a set of genes that are critical to their function. Further, it implies that the path of differentiation that stem cells might take is determined by factors supplied by the niche where they reside. A recent study compared the gene expression profiles of bone marrow derived and neural stem cells [11]. Remarkably, Terskikh et al found a group of transcripts that are shared by both stem cell populations including several that are membrane receptors with unknown functions. This suggests that when more stem cells are analyzed with this approach, a stem cell-specific complement of genes will be discovered that presumably will mediate the self-renewing property of these stem cells.

SELECTION VERSUS INSTRUCTION

The mechanism by which a progenitor cell enters a path of commitment leading to terminal differentiation is the central question in the development of all tissues and organs. Two ideas have been proposed: selection and instruction. In selection, the progenitor cell is seen as one whose fate is stochastically determined, or that its progeny (at least in a binary state) can be of one or another phenotype. The presumption is that the cell in one of its states already expresses some of the differentiated proteins. The factor that induces development then biases the outcome by selecting one of the progeny phenotypes, expanding this colony to lead to one type of fate. In the instructive mode, the factor induces the expression of new genes that mediate a specific program to force the cell into a specific differentiative path.

The discovery of the cytokines that mediate bone marrow development had suggested that specific factors such as erythropoietin, thrombopoietin and granulocyte colony-stimulating factor are all acting as instructive agents. However, recent studies where heterologous receptors or chimeric receptors were transfected into progenitors suggested that they are acting instead as selective factors [22]. While this may be true, it is important to decide *a priori*, what are the objects of regulation. Are they the cytokine factor or their receptors, or is it the signal transduction pathway and its presumed target, the master gene that activates the differentiation program? Many of the transfected factors or receptors that aim to distinguish between instruction and selection seem to activate the same or similar signal transduction pathways, and

hence the experiments mentioned above are not really decisive. Selection is a remarkably efficient mechanism to produce differentiated progeny and it has the added esthetic attraction of being the central mechanism of Darwinian evolution. However, there is no reason to think that all differentiation is going to be mediated by a single process. There are clear and well documented examples of selection and instruction available in various developmental systems, and it is the role of the investigator to identify which of these mechanisms apply at each stage of development of the tissue

EARLY EVENTS IN KIDNEY DEVELOPMENT

As is well known to readers of *Kidney International*, the mammalian kidney develops in three waves, only the last of which—the metanephros—continues as the adult kidney. The pronephros largely degenerates and only some remnants of the mesonephros remain in the adult to form the tubules of the rete testis and the vas deferens in the male. It is traditional to present the development of the adult kidney by stating that the metanephric kidney begins when an outgrowth of the wolffian (that is, mesonephric) duct, the ureteric bud invades the metanephric mesenchyme. Factors secreted by the ureteric bud induce this mesenchyme to first aggregate and then to convert to an epithelium. The induced mesenchyme in turn sends signals back to the ureteric bud to cause it to divide and grow. This reciprocal induction proceeds in an ordered manner to produce several generations of branches and eventually all the nephrons of the kidney. This critical process of mesenchymal induction with its cachet of conversion of one cell type to another has justifiably attracted much attention. The tip of each ureteric bud first induces the development of a renal vesicle, which undergoes morphogenetic transformation to eventually form the nephron from the glomerulus to the end of the distal tubule, while each ureteric bud eventually forms the collecting tubule.

All three embryonic kidneys arise from the dorsal mesoderm, a region that originates in the pre-axial mesoderm one of the earliest tissues to develop in the embryo. These mesenchymal cells have the ability to differentiate into epithelia. For instance, the development of the somites involves an initial conversion of mesenchyme to epithelia followed by later differentiation of these epithelia to the muscle and bone structures of each segment. It is customary to discuss kidney development as a special problem of formation of epithelial structures from the metanephric mesenchyme under the influence of the ureteric bud. However, the ability of mesenchyme to convert to epithelia is present in the forerunner of the metanephric mesenchyme. Indeed, the pronephros, an epithelial structure, also must develop by a process of mesenchyme to epithelial conversion. Finally, the origin of the wolffian duct also occurs by conversion of cells from the dorsal

mesoderm to the epithelium of the duct. These considerations become critical in deciding what is the locus of the stem cell that forms all the kidney structures. The fundamental issue is to decide where are we going to look for the stem cell that forms the kidney; will we look in the pre-somitic mesoderm, in the dorsal mesoderm or only in the metanephric mesenchyme?

The absence of a large body of knowledge regarding the role of stem cells in kidney developments has forced us to write this review to highlight urgent questions that need to be answered if our field is to build a deeper understanding of the cellular and molecular basis of kidney development and repair.

IS THERE A SINGLE PROGENITOR FOR ALL KIDNEY CELL TYPES?

One can answer this question half flippantly: it depends how early you look. More seriously, one has to define the earliest stem cell as one that can generate the cell types that populate the kidney and also state a corollary that no extrarenal cells are produced by this putative stem cell. Clearly, by this stringent definition we can exclude the pre-somitic mesoderm, since these cells will eventually produce the somites. What about the next region to differentiate, the intermediate mesoderm? Again, these cells produce other organs such as the gonads. Within this region is located the AGM (aorta-gonad-mesonephros), a region now known to be the major source of the hemopoietic stem cells that are destined to populate the liver (Fig. 3) [23]. With this in mind, it is possible that heterologous stem cells when isolated from their niche might still retain sufficient differentiative activity that they could produce renal cells. One study has already suggested that neural stem cells are capable of populating the mesonephros [24]. However, the question of whether there is a nephrogenic stem cell is still open. It is possible that cells exist in the intermediate mesoderm that are already committed to form a renal lineage including formation of the mesonephric duct and the mesenchyme that surrounds it, especially in the caudal region. Since we cannot at present answer the question posed in this section, let us first summarize what is known and then define the questions that may now be answered, at least tentatively. The wolffian duct generates the ureteric bud, the bladder and urethra. The ureteric bud generates all of the collecting tubules (cortical, medullary and papillary), and the pelvic and ureteric epithelia. The metanephrogenic mesenchyme generates the nephron epithelia. If we look at the two stages that bookend the process of kidney development we find that when kidney development begins (day 11 of embryonic life in the mouse), there must be at least four cell types in the embryonic kidney; let us call them: metanephrogenic mesenchyme epithelial cell precursors (MM-Ep),

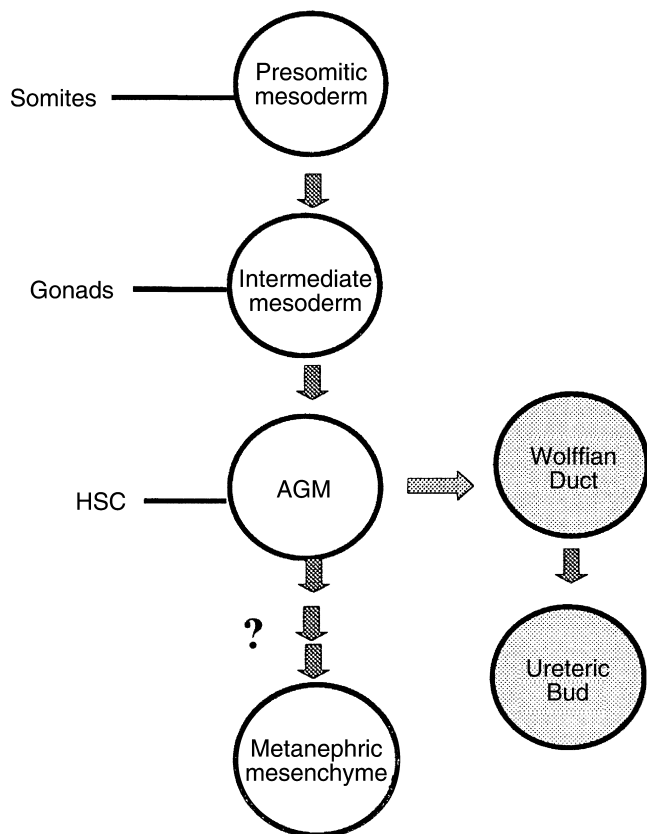


Fig. 3. Development of the metanephric mesenchyme.

angioblasts or endothelial cell precursors (MM-Ang), smooth muscle precursor (MM-SM), stroma cell precursors (MM-S), and ureteric bud cells (Fig. 4). As shown in Table 1, ten days later in the postnatal kidney of mice there are at least 26 terminally differentiated cell types, all recognizable by morphology, location and function. This number is likely to be underestimated, since recent studies have demonstrated that there are many subtypes of endothelial cells, and it is likely that glomerular and peri-tubular capillary endothelia (in the cortex and vasa recta) are fundamentally different. In addition, there is at present very little accounting or characterization of the interstitial types of cells that exist in the cortex and medulla.

How are all cells in the kidney related? While one hopes that in the near future it will be possible to construct such a "family tree," currently only the origin of the renal epithelial cells is understood. It has long been known that the ureteric bud generates the epithelia of the collecting duct, but it was unclear whether the epithelia of the rest of the nephron is derived from a single metanephric mesenchymal cell or whether each cell type had its own precursors. Recently, the presence of a single cell that can generate all the epithelial elements of the nephron was established using a lineage marker. A replication-defective retrovirus carrying a reporter gene was

used to infect cells from the induced metanephric mesenchyme at limiting dilution [25]. The progeny of the single infected cell were found to be located in all the epithelial regions of the nephron, including parietal and visceral glomerular epithelia, proximal tubule, loop of Henle and distal tubules. These studies show that a pre-epithelial compartment exists in the metanephric mesenchyme that is committed to generate all the epithelial cells of the different nephron segments excluding the collecting tubule. That is, the embryonic kidney contains mesenchymal cells that are renal epithelial stem cells (the putative MM-Ep).

More recently, Barasch et al have begun to lay the foundation for the identification of the molecular mechanisms of renal epithelial differentiation. They demonstrated that the metanephric mesenchyme contains clusters of cells destined to generate the epithelial cells of glomerulus, proximal and distal tubules [26]. These cells are programmed for apoptosis but were rescued from such fate by factors secreted by the ureteric bud such as bFGF [8] or tissue inhibitor of metalloproteinase-2 (TIMP-2) [26]. Following the rescue, it became clear that these cells acquire a certain recognizable phenotype in that they exist as clusters of cells that express *wnt 4* and other genes surrounded by stromal cells that express *tenascin*. Unexpected complexities were observed whereby the cells required factors not only from the ureteric bud to induce them to acquire the epithelial phenotype such as *LIF* [27], but also factors from surrounding stromal cells to allow the correct amount of balance between glomeruli and tubules.

What about the other cell types? Of these progenitor cell types, only the wolffian duct with of its characteristic location and developmental history has been well characterized. Of the other three, it is not clear whether they all derive from a single cell; even though Figure 4 is constructed to imply that this is the case, the dotted line emphasizes the ambiguity of their relatedness. Another possibility is that some cells derive from a renal stem cell and others invade the developing kidney from the outside, which is likely the mechanism for renal neuronal development, for example.

Perhaps because the renal circulation has such a central role in kidney function and disease, the potential origin of renal vascular cells attracted attention well before stem cells became an issue. Embryonic kidneys grown *in vitro* do not develop a vasculature. When mouse developing kidneys were grown on quail allantoic membranes glomeruli were vascularized by quail endothelia, suggesting that renal endothelial cells may be exogenous to the kidney. However, recent studies demonstrated that when embryonic kidneys are first grown *in vitro* and then grafted into the anterior eye chamber of a normal animal, the kidney develops endothelial cells (including glomerular capillaries) that are of graft origin [28]. These studies suggest that embryonic kidneys contain angioblasts the endothelial

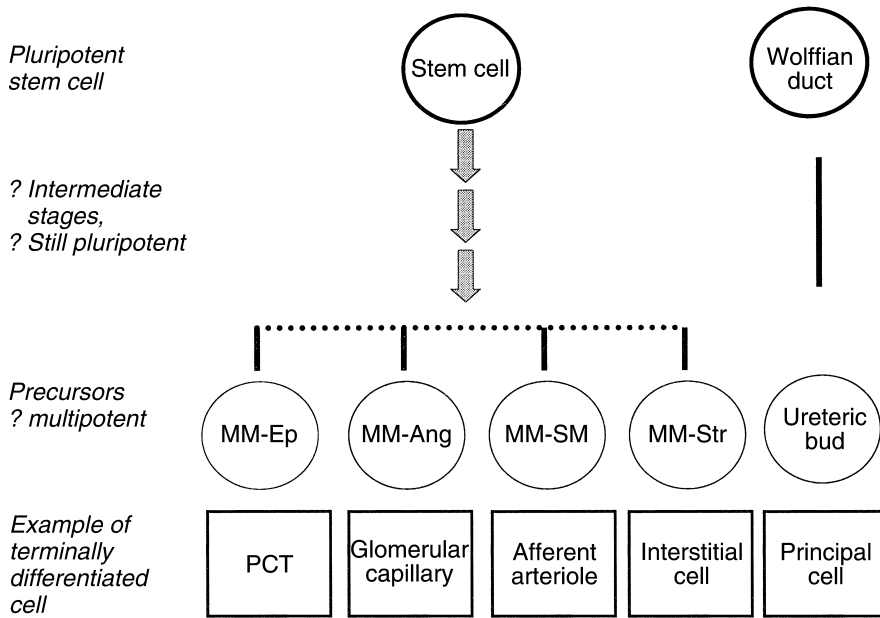


Fig. 4. A theoretical family tree of kidney cells. Abbreviations are: MM, metanephrogenic mesenchyme; MM-Ep, epithelial cell precursor; MM-Ang, angioblast; MM-SM, smooth muscle precursor; MM-Str, stromal cell precursor. Only one example of a terminally differentiated cell per precursor is shown. A more complete list is shown in Table 1.

Table 1. A catalogue of differentiated cells of the mature kidney

Nephron epithelia	Endothelia	Stroma	Smooth muscle	Wolffian duct	
				Ureteric bud	Urogenital sinus
Podocyte	Glomerular	Cortical	Mesangial	Principal	Bladder
Parietal	Peritubular	Medullary	Vascular	α -Intercalated	Urethra
Proximal	?Large vessel		Ureteric	β -Intercalated	
S1, S2, S3			Detrusor	Inner medullary	
Thin descending				Pelvis	
Thin ascending				Ureter	
Thick ascending					
Cortical					
Medullary					
Macula densa					
Distal tubule					

cell precursors (the putative MM-Ang). However, it is not clear whether these angioblasts invade the embryonic kidney from outside, are a population of cells distinct from the metanephric mesenchymal cells that will become renal epithelia, or derive from some distinct progenitor cell. Even less is known about the origin of the renal vascular smooth muscle and mesangial cells; during development they are generated much later than nephrons and capillaries. It is intriguing that the AGM region is one that will eventually form the aorta with its complement of endothelia and smooth muscle cells. Hence, the region that forms the kidney contains an abundance of endothelial and smooth muscle precursors.

In the experiment using a lineage marker described above [25], there were no labeled cells in the endothelium or in smooth muscle cells, suggesting that the infected cell either is too far down in its lineage restriction path or else the generation of endothelia and smooth muscle is produced by a different stem cell. However, recent work done in our laboratory with a cell line of

metanephric mesenchymal cells and primary cultures derived from E13 rat kidney mesenchyme indicate that these cells are pluripotent and can differentiate into myofibroblasts, smooth muscle, and, perhaps also into endothelial cells in addition to epithelia (abstract; Oliver JA, *J Am Soc Nephrol* 11:380A, 2001). If confirmed in vivo, these results suggest that renal epithelial stem cells in the embryonic kidney have a broader differentiation potential than previously thought and thus are pluripotent renal stem cells. Characterization of these cells may provide information useful in isolation and characterization of stem cells in the adult kidney.

WHEN DOES COMMITMENT TO A SPECIFIC DEVELOPMENTAL PATHWAY OCCUR?

Initial studies where we labeled single cells in the induced mesenchyme showed that the infected cells were committed to an epithelial lineage. Infection of the induced mesenchyme at a later stage of development demonstrated

that the progeny of the cells now could populate only one segment of the nephron, for instance, glomerulus or proximal tubule, thereby demonstrating the presence of lineage restriction. However, since the infection was performed in a “blinded” manner, it is not possible to identify the type and stage of the cell that was being infected. Restriction occurs in other kidney lineages, since we found that shortly after the invasion of the ureteric bud, many metanephrogenic mesenchymal epithelial precursors contain markers characteristics of endothelial phenotype, but that as development progresses these markers are restricted to fully differentiated endothelial cells (unpublished observations).

These results suggest that lineage restriction occurs at some stage during the development of the kidney, but strictly speaking we are not able to exclude the extreme condition that the metanephric mesenchyme contains many lineage restricted cells even before the invasion of the ureteric bud, and that further development is due to the selection of these cells by a cascade of locally-produced growth factors produced by the branches of the ureteric bud or by other cells such as the stroma. We need to develop criteria whereby each of the stages shown in Figure 4 is identified and their characteristic gene expression pattern determined. From this kind of catalog we will then be able to determine whether these cells are truly lineage restricted. These studies will require clonal cultures to demonstrate the fate of each cell *in vitro*. Needless to say, one has to supplement these studies by others where the cells are marked at that stage *in situ*; that is, in their original niche, and determine whether the *in vitro* studies truly reflect the *in vivo* pattern.

ARE THERE STEM CELLS IN THE MATURE KIDNEY AND IF YES, WHERE DO THEY RESIDE?

It is well known that the bone marrow, small intestine, skin, cornea and prostate of adult animals contain cells able to regenerate the cell types of that organ, and more recent studies have suggested that many and perhaps all adult organs harbor stem cells. Hence, we expect that some cells in the mature kidney will turn out to be resident stem cells. Which methods can be used to identify and locate them? Because organ-specific adult stem cells have the ability to regenerate adult tissue and divide very slowly, one way to locate them has been to follow cell division and identify a group of slow cycling cells before and after an injury. For example, in the skin and cornea, a wound forces stem cells in the “bulge” and limbus respectively to enter the cell cycle. Similarly, analysis of the recovery phase of acute tubular necrosis may provide clues as to location and identity of renal stem cells. Many investigators have noted that several cells begin to divide during the recovery from acute tubular

necrosis. These were tubular epithelial cells that seemed to be the cells next to the injured ones. In unilateral ureteral obstruction, several interstitial cells also have been found to divide rapidly. These studies did not aim to characterize the locus of stem cells in the adult kidney, and thus the level of characterization currently does not permit a conclusion about where the slow cycling compartment was located or even whether it was present. Another possible method to identify adult renal stem cells would be to try to locate in adult kidney a population of cells with the phenotype of metanephric mesenchyme epithelial precursors, cells that on transplantation into developing kidneys can generate all nephron epithelia and smooth muscle.

Finally, one may entertain more speculative methods of stem cell location and identification. Embryonic nephrogenesis occurs at a stage in which the circulation is not yet established and oxygen tension is low, raising the possibility that hypoxia is at least permissive for kidney stem cell functions. It is tempting to speculate that a hypoxic compartment in the adult kidney such as the medulla may provide a niche that allows a population of renal cells to maintain their “stemness.” Incidentally, since the kidney grows “centrifugally,” the medulla is the oldest region of the kidney and hence might be the one that contain the rest of the stem cells.

WILL KIDNEY STEM CELLS PERFORM IN VITRO THE SAME FUNCTIONS AS THEY DO IN THEIR NICHE?

The field of stem cell research has been dominated by ideas generated by the hemopoietic system. A single cell can be isolated from peripheral blood, be grown in suspension, and form all the components of the blood and lymphoid tissues. This dominance extends to the manner in which the process is displayed graphically (Figs. 2–4). Other organs are three-dimensional structures where during embryonic development the position of every cell exposes it to a morphogenetic field of specific shape and intensity. These morphogens often are produced or neutralized by other cells. Hence, the question of whether a stem cell in culture will produce the same progeny as *in situ* remains unanswered. Indeed, even for bone marrow stem cells, it seems that injection of these cells into the peripheral blood is immediately followed by homing of these cells to the bone marrow where they prefer to reside, suggesting that the niche confers some advantage. Therefore, while it may be possible to isolate a single stem or multipotent cell and define its requirement for conversion to one or another lineage, these studies ought to be supplemented with injection of the cell into developing kidneys to allow observation of its “natural” development. The availability of strains of mice that ubiquitously express the β -galactosidase gene (ROSA mice)

should facilitate these studies. In addition, the presence of continued nephron development after birth in mice has an added benefit in allowing studies on animals larger than embryos.

CONCLUSIONS

At the time that the developing kidney first becomes an anatomical entity it is made up of the metanephrogenic mesenchyme and the invading ureteric bud. It has long been known that the cells of the ureteric bud are the precursors of the epithelial cells of the collecting duct. The metanephrogenic mesenchyme contains precursors for the epithelial cells of the rest of the nephron, endothelial cell precursors and stroma cells, but the exact relatedness between these cells is unclear. Because a single metanephric mesenchymal cell can generate any epithelial cell along the nephron (excluding the collecting duct) it is apparent that the embryonic kidney contains epithelial stem cells. We have recently found that, at least in vitro, these cells also can differentiate into myofibroblasts, smooth muscle and, likely, endothelial cells. If these results are confirmed in vivo, they would indicate that metanephrogenic mesenchyme epithelial precursors are pluripotent kidney-specific embryonic stem cells. Whether the adult kidney contains stem cells is unknown, but work in other organs suggests that this is the case and lessons from other tissues and renal development may help to identify them. For example, genes shared by organ-specific stem cells from different organs, as recently identified in hemopoietic and neural stem cells, might provide definitive markers. Alternatively, identification in the adult kidney of cells with phenotypic characteristics of stem cells (such as, proliferation with slow division time following injury) also may prove fruitful. Finally, because renal stem cells are present at the beginning of renal development, detailed study of early renal embryonic cells and their immediate progeny may provide important information. Identification and characterization of adult renal stem cells is likely to radically change our understanding of kidney development, function and repair, and hopefully will lead to new and effective therapies, sorely missing for most renal diseases. Beyond doubt, this is currently the most pressing problem in nephrology.

Reprint requests to Qais Al-Awqati, M.D., Ch.B., Department of Medicine, College of Physicians and Surgeons of Columbia University, 630 W. 168th Street, New York, New York 10032, USA.
E-mail: qa1@columbia.edu

REFERENCES

- WEISSMAN IL: Stem cells: units of development, units of regeneration, and units in evolution. *Cell* 100:157–168, 2000
- MORRISON SJ, WHITE PM, ZOCC C, ANDERSON DJ: Prospective identification, isolation by flow cytometry, and in vivo self-renewal of multipotent mammalian neural crest stem cells. *Cell* 96:737–749, 1999
- ANDERSON DJ: Stem cells and pattern formation in the nervous system: The possible versus the actual. *Neuron* 30:19–35, 2001
- LU B, JAN L, JAN YN: Control of cell divisions in the nervous system: symmetry and asymmetry. *Annu Rev Neurosci* 23:531–556, 2000
- COTSARELIS G, CHENG SZ, DONG G, *et al*: Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: Implications on epithelial stem cells. *Cell* 57:201–209, 1989
- TAYLOR G, LEHRER MS, JENSEN PJ, *et al*: Involvement of follicular stem cells in forming not only the follicle but also the epidermis. *Cell* 102:451–461, 2000
- VICARIO-ABEJON C, JOHE KK, HAZEL TG, *et al*: Functions of basic fibroblast growth factor and neurotrophins in the differentiation of hippocampal neurons. *Neuron* 15:105–114, 1995
- BARASCH J, QIAO J, MCWILLIAMS G, *et al*: Ureteric bud cells secrete multiple factors, including bFGF, which rescue renal progenitors from apoptosis. *Am J Physiol* 273:F757–F767, 1997
- LAVKER RM, SUN TT: Epidermal stem cells: Properties, markers and location. *Proc Natl Acad Sci USA* 97:13473–13475, 2000
- UCHIDA N, BUCK DW, HE D, *et al*: Direct isolation of human central nervous system stem cells. *Proc Natl Acad Sci USA* 97:14720–14725, 2000
- TERSKIKH AV, EASTERDAY MC, LI L, *et al*: From hematopoiesis to neurogenesis: Evidence of overlapping genetic programs. *Proc Natl Acad Sci USA* 98:7934–7939, 2001
- BJORNSEN CR, RIETZE RL, REYNOLDS BA, *et al*: Turning brain into blood: A hematopoietic fate adopted by adult neural stem cells in vivo. *Science* 283:534–537, 1999
- ASAHARA T, MUROHARA T, SULLIVAN A, *et al*: Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275:964–967, 1997
- FERRARI G, CUSELLA-DE ANGELIS G, COLETTA M, *et al*: Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 279:1528–1530, 1998
- PETERSEN BE, BOWEN WC, PATRENE KD, *et al*: Bone marrow as a potential source of hepatic oval cells. *Science* 284:1168–1170, 1999
- EGLITIS MA, MEZEY E: Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. *Proc Natl Acad Sci USA* 94:4080–4085, 1997
- ORLIC D, KAJSTURA J, CHIMENTI S, *et al*: Bone marrow cells regenerate infarcted myocardium. *Nature* 410:701–705, 2001
- BAROFFIO A, HAMANN M, BERNHEIM L, *et al*: Identification of self-renewing myoblasts in the progeny of single human muscle satellite cells. *Differentiation* 60:47–57, 1996
- GUSSONI E, SONEOKA Y, STRICKLAND CD, *et al*: Dystrophin expression in the mdx mouse restored by stem cell transplantation. *Nature* 401:390–394, 1999
- ANDERSON DJ, GAGE FH, WEISSMAN IL: Can stem cells cross lineage boundaries? *Nat Med* 7:393–395, 2001
- KRAUSE DS, THEISE ND, COLLECTOR MI, *et al*: Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* 105:369–377, 2001
- SOCOLOVSKY M, LODISH HF, DALEY GQ: Control of hematopoietic differentiation: Lack of specificity in signaling by cytokine receptors. *Proc Natl Acad Sci USA* 95:6573–6575, 1998
- MEDVINSKY A, DZIERZAK E: Definitive hematopoiesis is autonomously initiated by the AGM region. *Cell* 86:897–906, 1996
- CLARKE DL, JOHANSSON CB, WILBERTZ J, *et al*: Generalized potential of adult neural stem cells. *Science* 288:1660–1663, 2000
- HERZLINGER D, KOSEKI C, MIKAWA T, *et al*: Metanephric mesenchyme contains multipotent stem cells whose fate is restricted after induction. *Development* 114:565–572, 1992
- BARASCH J, YANG J, QIAO J, *et al*: Tissue inhibitor of metalloproteinase-2 stimulates mesenchymal growth and regulates epithelial branching during morphogenesis of the rat metanephros. *J Clin Invest* 103:1299–1307, 1999
- BARASCH J, YANG J, WARE C, *et al*: Mesenchymal to epithelial conversion in rat metanephros is induced by LIF. *Cell* 99:377–386, 1999
- ABRAHAMSON DR, ROBERT B, HYINK DP, *et al*: Origins and formation of microvasculature in the developing kidney. *Kidney Int* 54 (Suppl 67):S7–S11, 1998