APRIL 2008

Stem Cell Science:

Overviews of Selected Disease Areas

Understanding the Development and Repair of the Kidney

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The Harvard Stem Cell Institute (HSCI) is a scientific collaborative established in 2004 to fulfill the promise of stem cell biology as the basis for the cure and treatment of a wide range of chronic diseases and medical conditions. HSCI's unique effort unites experts across the disciplines, schools and departments of Harvard University and all its affiliated research hospitals.

HSCI also sponsors public education programs concerning the scientific, legal and ethical implications of stem cell research, conducts a summer research program for college students, and helps educate high school teachers about stem cell science. HSCI depends upon the vision and generosity of private individuals, foundation and corporate donors to carry on its work, due to current U.S. restrictions on federal funding of embryonic stem cell research.

Introduction

The following scientific overview focuses on the use of stem cells-both in research and potential therapeutic applications - to address one of the most challenging, life-changing diseases and conditions of our time. This overview along with its companion pieces has two educational objectives: to make clear the opportunities and promise inherent in the basic science and to provide you with a picture of the research avenues that must be pursued to reach clinical applications. The overviews point to the areas where fundamental questions exist, where therapies need improvement, and where funding for research is urgently required.

Immense hope and scientific effort at institutions like the Harvard Stem Cell Institute have been invested in stem cells. Researchers seek the fullest understanding of how cells' fates are determined. They want to know how embryonic stem (ESC) cells recognize and respond to the signals that move them to become the full range of mature cells in an animal. They want to know how adult stem cells, whose fates are more restricted, contribute to the repair and regeneration of organs and tissues. Studying both types of cells in parallel will provide us information pertinent to developing stem cell therapies. With each experiment and clinical trial, researchers and clinicians move closer to these goals. This set of papers summarizes the current state of stem cell science in several key disease areas.

Stem cell research adds its own unique challenges to those faced by any early stage science. In fact, there are many cells, along with their behavior, that are still being discovered. In some cases the benefit, whether detailed fundamental knowledge, development of new drugs, or transplantable cells, is likely to be far in the future; in other cases, progress will come amazingly quickly. In stem cell research, as in few other contemporary scientific enterprises, success depends on supportive collaboration among diverse constituents: scientists and clinicians; local, state, and federal governments; universities and industry; and patients and their advocates. As a cross-institutional collaborative research and educational organization, HSCI is committed to meeting this challenge. We hope these disease overviews will help you better understand the research areas that matter to you.

I would welcome your thoughts about these overviews, your questions, and concerns. Contact me at brock_reeve@harvard.edu.

Brock Reeve, MPhil, MBA
Executive Director

Understanding the Development and Repair of the Kidney

Introduction

Identifying and understanding the cellular processes underlying development and repair of the mammalian kidney may enable more effective, targeted therapies for acute and chronic kidney diseases in humans. Given that thousands of people each year are affected, that their quality of life is dramatically diminished and that the related cost to America's health-care system runs in many billions of dollars, the potential rewards are significant.

In many organ systems, especially those with particularly high cellular turnover such as blood, skin and gut, stem cells are the basic currency of organ repair. What of the kidney? The kidney has a remarkable ability to survive injury and restore its function. Are stem cells called upon to rerun developmental programs in injured adults? Or is function restored and cellular damage repaired by some other process? Why can adult fish still generate new nephrons whereas mammals repair only those nephrons formed during fetal life?

More is known about how the kidney is formed than many other organs, but much remains to be discovered. By combining techniques of genetic manipulation in mice with large-scale study of DNA and its associated proteins (high-throughput genomics and proteomics), researchers are making rapid progress in linking cellular and organic responses to development and maturity. Several types of kidney cells have been proposed as adult stem cells and are now being scrutinized. The challenge at hand is finding genetic markers that will allow us to conclusively identify the progenitor cell populations in the fetal kidney, to trace their lineage and growth, to analyze molecular and cellular processes that govern these events, to determine how these relate to adult repair processes, and to understand how developmental insight might be used to design de novo repair processes.

The Kidney

The mammalian adult kidney filters the entire volume of a human body's blood every 20 minutes. It removes toxins and metabolic wastes and maintains the balance of water, salts and pH necessary to sustain life. Each kidney has approximately a million nephrons—long, convoluted epithelial tubes (see Figure 1) that act as tiny filtering units inside the kidney. During development, a complex dialogue mediated by molecular signals orchestrates the assembly of hundreds of thousands of nephrons from the resident progenitor cell populations (cells that are at an immature stage and can develop into mature adult cells). Several steps of this process are known, but we have only a partial picture of the interplay of gene regulation that prompts the creation of the kidney.

Fully understanding the cells involved and the signaling pathways will help us understand how to ward off congenital defects and suggest new approaches to manipulating kidney function in adults. As blood enters the kidney and travels along the various specialized domains of the nephron, essential nutrients, salts and water are returned to the blood while nitrogenous waste is removed in urine that passes from the kidney to the bladder. This maintains a healthy balance of useful chemicals and waste materials. In addition, kidneys release important hormones for red blood cell formation, the regulation of blood pressure, and the maintenance of calcium.

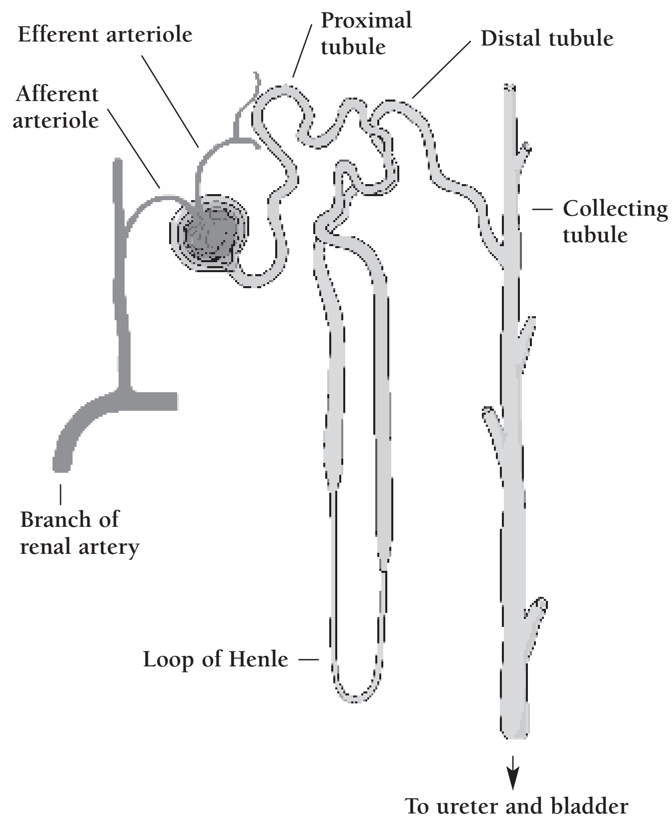

FIGURE 1:
A nephron. Source: www.arthursclipart.com

Key questions about kidney development and repair include:

- Do adult kidney stem cells exist, and if so, what is their role?
- What are the cellular and molecular mechanisms of the response to injury?
- How can we use what we know about kidney development to better understand the response to injury in an adult kidney and vice versa?

Regeneration and Response to Kidney Injury

Characterizing the role of stem cells in the adult kidney is at the hub of current renal stem cell research. In this type of research, scientists use mouse models to deepen their understanding of the impact of disease and injury. Within hours of an ischemic injury to the mouse kidney (caused experimentally by briefly interrupting blood flow), injured cells die and slough off the tubule's underlying basement membrane. (See Figure 2.) Surviving cells appear to return to a more immature state, as indicated by the expression of cell adhesion proteins such as vimentin, neural cell adhesion molecule (N-CAM), basic fibroblast growth factor (b-FGF) and Paxillin-2 (Pax-2), all hallmarks of the cell's normal progress from embryo to finished organ. These cells proliferate rapidly, migrating to cover areas of exposed basement membrane. Several observations have led to the speculation that kidney stem cells exist: the apparent recapitulation of some developmental processes during kidney repair, evidence that new epithelial precursors are in an undifferentiated state and divide rapidly, and the discovery of cells close to the kidney tubules that share features in common with stem cells in other organ systems. Still remaining is the question of whether there are kidney stem cells in the developing embryo and/or the adult organism.

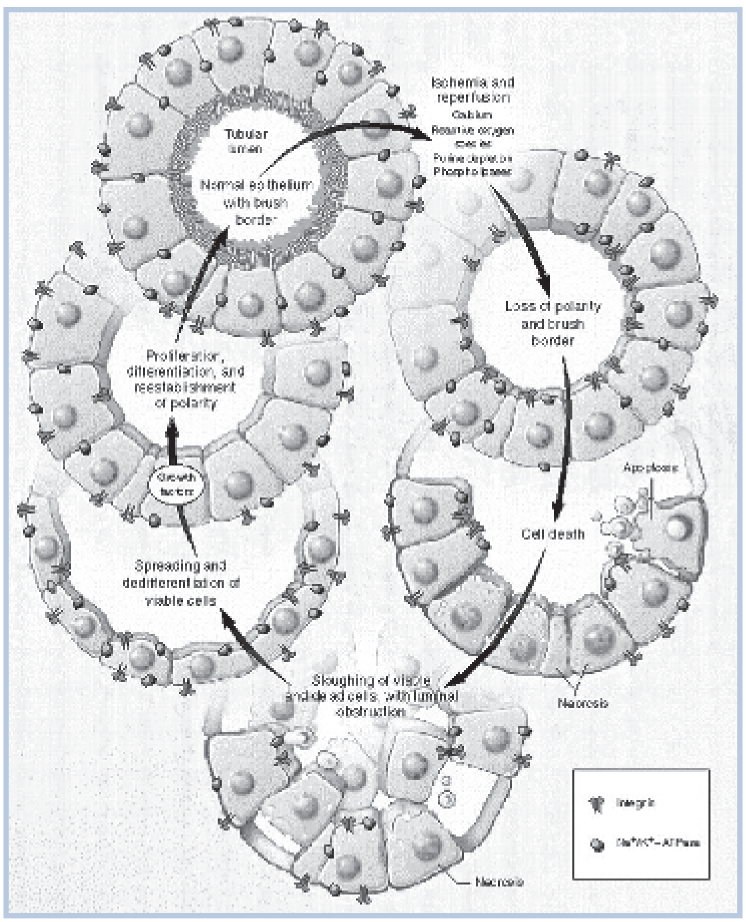

FIGURE 2:
Proximal tubule epithelium response to injury. An experimentally induced ischemia and reperfusion of blood disrupts the Na-K ATPase, which maintains the ionic gradient across the tubule. A portion of epithelial cells die, either from necrosis or programmed cell death (apoptosis). Dead cells and some live cells slough off the basement membrane. Dedifferentiated cells proliferate and spread over denuded areas, restoring a fully functional epithelium. Source: Thadani, *et al. N Eng J Med.* 334:1448-1460, 1996.

Recent studies in a developing fetus have identified distinct self-maintaining cell populations that work together to generate nephrons. Whether these are transient cells unique to the developmental program or persist as stem cells in the adult is a critical issue. To date, the search for renal stem cells in adult kidneys, both with and without injury, has produced ambiguous results. Studies have identified possible populations of stem cells in renal tubules, the glomerulus, interstitial tissue and the renal cortex. Some especially intriguing clues point to a stem cell population in the renal papilla, where collecting tubules join to form the ureter. These cells have common qualities with other known adult stem cells: They are physically segregated into physical supportive microenvironments, called niches, and they divide slowly. These two factors, combined with other possible molecular mechanisms for protection, shield stem cells from external stimuli, helping them to survive injury.

Understanding how new epithelium is generated and what types of cells give rise to it are keys to understanding renal injury in a clinical capacity. Without definitive genetic markers, however, tracing the maturation pathways of possible renal stem cells in the papilla, or anywhere else, is difficult, as most approaches are indirect. Current experiments that genetically mark distinct regions of the nephron, or the progenitor cell types in the developing kidney, offer hope that these questions can be resolved. If new epithelia can be mobilized even more rapidly or augmented by an outside influence, an injury could be less severe and recovery quicker.

Kidney Stem Cell Differentiation in Development

In concert with studies into epithelial regeneration following injury, investigators are also unraveling the cellular lineage and signaling pathways that occur during the development of the nephron and the kidney as a whole. In addition to answering fundamental questions about development, these studies could identify molecular and cellular processes that are recapitulated after an injury in the adult and the genetic markers for these processes, which could then be targets of new therapies.

In mammals, nephrogenesis (see Figure 3) depends on a reciprocal sharing of signals between two embryonic tissues, the ureteric bud and the metanephrogenic mesenchyme, which surrounds the ureteric bud. As signals are exchanged, the ureteric bud grows into the mesenchyme. It branches and organizes clusters of mesenchymal cells to condense at its tips and guides the differentiation and integration of those cells to produce the nephron. What cells constitute the metanephrogenic mesenchyme? How do these cells interact with one another and the mature structures of the adult kidney? What factors maintain these progenitor cells in their uncommitted state, and what factors induce progenitors to differentiate into nephrons?

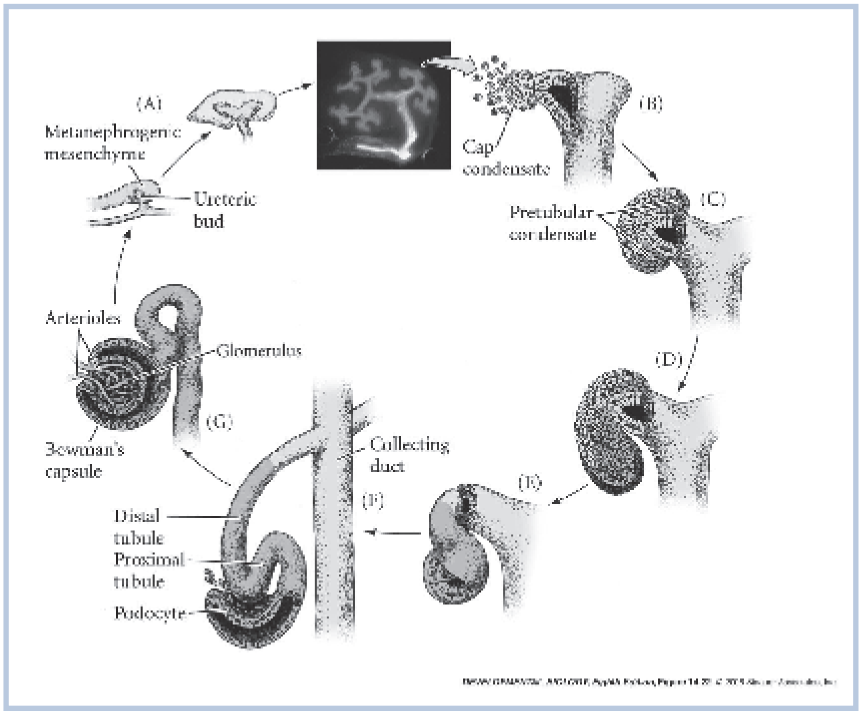

FIGURE 3:
Nephron development. Reciprocal signals between the metanephrogenic mesenchyme and the ureteric bud causes mesenchymal cells to aggregate around the bud and differentiate into the glomerulus and tubules. The bud becomes the collecting duct. Source: Gilbert, *Developmental Biology, 8th ed.,* 2006

More is known about kidney development than many other organs, and several of the signaling molecules or genes required for development have been identified. One example, Wnt9b, is a crucial protein secreted by the ureteric bud specifically to act on mesenchymal nephron progenitors. Wnt9b can induce these progenitors to aggregate and transition into precursors of the primary epithelial network of the nephron. Although we understand how Wnt9b signals are received, we still need to determine what molecular targets are critical to this signaling pathway.

Researchers have identified an enzyme, telomerase (TERT), that regulates telomere length. (Telomeres are regions at the end of eukaryotic chromosomes that shorten each time a cell divides; this has been implicated in aging.) TERT may be a marker for mesenchymal cells destined to become tubule cells. Researchers are using this and other markers to ask questions such as whether additional mesenchymal cells are added to the tubule-generating population or if the pool is maintained by proliferation of existing tubule progenitors. New information about genetic markers for different renal cell types can be used in studies of regeneration following injury.

What Can We Do with Kidney Stem/Progenitor Cells?

Identifying and fully characterizing stem/progenitor cells in the kidney during embryonic development and in adult tissue would have immediate implications for clinical practice:

- Enhancing the body's response to epithelial injury or death could lessen the severity of acute kidney disease, shorten recovery time and perhaps lessen the likelihood that a patient will develop chronic kidney disease.
- The cells could be targets for therapies to combat not just acute kidney disease but also chronic and degenerative diseases, including congenital disorders of the kidney where tissue generation or regeneration is needed.
- Cell lines carrying specific kidney diseases would accelerate research into renal pathology as well as provide a system to evaluate molecules or protocols for their therapeutic potential.
- Kidney stem cells, grown and differentiated in vitro, could be incorporated in next-generation dialysis devices. Unlike the mechanical and chemical systems now in use, these extracorporeal devices would provide more effective replacement for failed kidney function, since they would employ the natural biological mechanisms of the kidney.

Despite the abundant promise of progress, there remains the essential challenge in understanding the role of stem cells in the kidney: the contrast between positively identified stem cells in the developing organism and the very existence of stem cells in adults. Identifying and characterizing the role of stem cells in the adult kidney is a significant component of renal research that must move forward before approaches to therapy and repair can be fully realized.

For more information about the HSCI Kidney Disease Program, visit the HSCI Web site at **www.hsci.harvard.edu**.

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