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Label-retaining cells in the kidney: origin of regenerating cells after renal ischemia

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Abstract
The kidney is capable of regeneration. In response to a variety of insults, renal epithelial tubular cells dedifferentiate into an immature phenotype, proliferate, migrate to the injured area, and redifferentiate into mature polarized epithelial cells. In animal models of acute kidney injury induced by renal ischemia or renal toxins, various growth factors, transcription factors, chemokines, and extracellular matrix components have been demonstrated to be involved in the regeneration process. Recent research has suggested the existence of renal stem/progenitor cells in the kidney and their involvement in renal regeneration. In this review, we will focus on the mechanisms of tubular regeneration after kidney injury, particularly on label-retaining cells actively engaged in this process, and discuss their potential as targets of regenerative therapy for various kidney diseases.

Key words: Label-retaining cell · Tubular regeneration · Renal stem cell · Activin-follistatin system · Kidney development

Introduction
The kidney undergoes continuous, slow cellular turnover for tissue maintenance and rapid cell replacement after injury. However, once kidney damage is established, it cannot be reversed or repaired by currently available treatments, and renal function deteriorates to renal failure, which is often life-threatening. The number of patients requiring hemodialysis is increasing annually.

Recently, as with other tissues, research regarding regenerative therapies, such as the development of new strategies for improving the degree of recovery following injury, has drawn much attention in the field of nephrology. Recent evidence suggests that hematopoietic stem cells (HSCs) or mesenchymal stem cells (MSCs) contribute to kidney regeneration after injury. Transplantation of HSCs or MSCs has been demonstrated to reduce renal injury in an ischemia/reperfusion injury model, although the mechanisms by which these HSCs or MSCs enhance renal repair remain unknown. Factors produced by HSCs or MSCs appear to exert renoprotective effects rather than showing direct differentiation into functional tubular cells; nonetheless, HSCs or MSCs are considered to be promising sources of regenerative cell therapy for various kidney diseases. Embryonic stem (ES) cells are also an attractive cell source for tissue engineering. Ex vivo reconstruction of functional kidneys or tubules from ES cells has been investigated, although such research is still in the preliminary stages. The ultimate goal of reconstituting an entire kidney in vitro may be impossible due to the complexity of the tissue. However, these approaches will be needed for the development of new strategies for the treatment of kidney diseases. Whether renal stem/progenitor cells exist in the kidney is of great interest, as they may ultimately have important therapeutic applications. Renal stem/progenitor cells could be therapeutic targets to modulate tissue remodeling in chronic kidney disease, either by promoting their activation or preventing their depletion.

In this review, we will summarize our data regarding the regenerative process of the kidney after injury, with particular focus on label-retaining cells (LRCs), a cell population actively engaged in this process, and we will discuss the potential of these cells as targets for the treatment of chronic kidney disease.

Regeneration process of the kidney after injury
Tubular cells are an important cell type in the kidney. The degree of tubular damage is directly related to the impairment of renal function. Understanding the tubular repair process is indispensable for developing new strategies for
the treatment of kidney disease. It is thought that, in acute renal failure, tubular recovery is mediated by the surrounding tubular cells that border the region of injury. After the insult occurs, these cells rapidly lose their brush border and dedifferentiate into a more mesenchymal phenotype. The dedifferentiated cells migrate into the regions where cell necrosis, apoptosis, or detachment has resulted in denudation of the tubular basement membrane. There, they proliferate and eventually redifferentiate into an epithelial phenotype, completing the repair process. In animal models of acute kidney injury induced by renal ischemia or renal toxins, various growth factors have been found to be involved in tubular regeneration after injury. The local release of growth factors is thought to be triggered by renal tubular cell stress or injury. These growth factors are considered to mediate the migration, dedifferentiation, and proliferation of surviving cells at sites of tubular injury, and also to regulate cell maturation and restoration of polarity, allowing the organ to return to its basal physiological role as regulator of the internal milieu. Extracellular matrix (ECM) molecules and ECM-related proteins are also known to coordinate this process. They provide a physical substrate for the spatial organization of the cells, act as a potential reservoir for growth factors, and regulate signal transduction pathways by binding to various integrins.

Recapitulation of the developmental paradigm in kidney regeneration

The regeneration process is thought to resemble its developmental paradigm. The remodeling and maturation of restored epithelium after renal injury have many parallels with the growth and maturation that occur during kidney organogenesis. Various growth factors critical for renal organogenesis have been shown to be involved in kidney regeneration and to play important roles in this process as mitogens, motogens, and morphogens.

Consistent with this notion, activin A, a developmental molecule involved in renal organogenesis, has been found to be involved in kidney regeneration after injury. Activin A is a member of the transforming growth factor (TGF)-beta superfamily, and is known to regulate cell growth and differentiation in various tissues. In an in vitro Wolffian duct (WD) culture system, activin A was found to negatively regulate ureteric bud (UB) outgrowth from the WD. Activin A also inhibits UB branching, and is required for metanephric mesenchyme (MM) differentiation during kidney development. In the kidneys of transgenic mice expressing truncated activin type II receptor, the number of glomeruli was significantly increased when compared to that in wild-type mice. Collectively, it is possible that activin A acts as a negative regulator of renal organogenesis. This notion was supported by the results obtained from an in vitro tubulogenesis model using Madin-Darby canine kidney (MDCK) cells. Hepatocyte growth factor (HGF) induced branching tubulogenesis, which was inhibited by activin A. Interestingly, blockade of activin action by follistatin or overexpression of activin mutant receptor induces tubulogenesis, even in the absence of HGF, thus suggesting that activin A is an autocrine inhibitor of branching tubulogenesis. This cell culture system can be viewed as a basic approach supporting tubular engineering. Although it is unknown whether these in vitro tubules would be functionally mature, the activin-follistatin system might be an important regulatory system for developing artificial tubules.

Recently, we investigated the role of the activin-follistatin system in the tissue repair and regeneration processes of the kidney, using an ischemia/reperfusion injury model. Expression of activin increased significantly in the kidneys after ischemic injury. In contrast, expression of follistatin, abundant in tubular cells of the outer medulla of normal kidneys, decreased significantly after renal ischemia. Expression of the activin receptors was also detected in tubules of normal kidneys. Exogenous follistatin was found to alleviate renal damage after ischemic injury, probably by promoting proliferation and reducing apoptosis in tubular cells. Furthermore, reactivation of Pax-2, a key regulatory molecule during renal organogenesis, was observed in the nuclei of regenerating tubular cells after ischemic injury. The number of Pax-2-positive cells in ischemic kidneys was increased after the administration of follistatin and, conversely, was reduced after the administration of activin A. Inactivation of activin signaling pathways also induced the upregulation of Pax-2 expression in a renal tubular epithelial cell line. Thus, the activin-follistatin system may modulate renal regeneration after injury by controlling Pax-2 expression. The blockade of endogenous growth inhibitors may be an alternative method for accelerating renal regeneration, instead of growth-promoting factor infusion.

Label-retaining cells (LRCs)

Stem cells generally possess an inexhaustible capacity for self-renewal and differentiation to ensure the lifelong maintenance of tissue homeostasis. Stem cells have a slow turnover and display minimal physiological differentiation. To conserve growth potential and prevent genetic injury during mitosis, stem cells are thought to cycle slowly and are recruited only as demanded by tissue turnover. As early descendents of stem cells, transit-amplifying (TA) cells retain significant growth capacity while acquiring differentiated functions. TA cells eventually become incapable of proliferation and enter a terminally differentiated state.

One of the most common methods to identify stem cells is to search for slow-cycling cells by labeling their DNA. A pulse of 5-bromo-2′-deoxyuridine (BrdU) mostly labels TA cells. Long-term BrdU labeling is thought to mark stem cells that retain the label for an extended period as a result of slow turnover. Adequate labeling intensity and a suitable washout period for the TA and terminal differentiation compartments will thus result in so-called LRCs, which are believed to represent the stem cell compartment.

Utilizing this design, LRCs have now been reported in many adult organs. These include the lung, pancreas,