Kidney Regeneration and Stem Cells

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Organs are formed during embryogenesis by proliferation and differentiation of stem cells. This ability of an organism to form new tissue persists to varying degrees during the postnatal life. Lower animals such as planarians can regenerate an entirely new organism from a small body segment; while humans are merely able to restore organ structure following injury. With the identification of stem cells in many adult organs including kidneys, scientists are developing novel therapies for facilitating organ regeneration. Presently, mesenchymal stem cells (MSCs) have emerged as the most promising stem cell type for facilitating kidney regeneration following acute injury. In an initial clinical trial, MSCs prevented and ameliorated acute kidney injury, and patients tolerated MSC administration well without significant adverse effects. MSCs are currently undergoing further clinical testing before becoming a standard of care. Further examples of such innovative therapies include renal assist device; refurbishing kidney scaffolds with new cells; formation of new functioning units of kidney in a synthetic matrix; and transplantation of embryonic kidneys in adult animals. Although most of these therapies are still in the early stages of development, they will require advancement of technology before they become applicable for treating human kidney diseases. These proof-of-principle experiments advance these innovative therapies into a potentially achievable category and provide true hope to patients with kidney disease.

Key Words: Kidney Regeneration; Stem Cells; Progenitor Cells; Bioartificial Kidney

Introduction

Organ regeneration following injury is one of the most intriguing fields in the modern medicine that has rapidly evolved in the past decade. Organ regeneration recapitulates development and varies greatly among different species. For example, invertebrates such as planarians show high degrees of regeneration and can regenerate entire organisms from small body segments following amputation. In contrast, vertebrates have limited regenerative ability and can regenerate only amputated body parts to varying degrees, but not the entire organism. Frequently, vertebrates lose their regenerative ability as adults. For example, Anuran tadpoles can regenerate limbs only before metamorphosis, while newts and axolots retain the regenerative ability throughout larval and adult life [1].

Similar to limb regeneration, regeneration following partial resection of an internal organ, such as the kidney, is variable among different species. Lower animals such as cartilaginous fish can form new nephrons—the functioning units of kidney—following injury; while higher animals merely demonstrate compensatory growth of the remaining nephrons, which is known as “regenerative hypertrophy” [2]. The ability to form new nephrons is often called “neonephrogenesis”. Elasmobranchs, such as skate and dogfish, demonstrate neonephrogenesis as adults following partial resection of kidney. Elger et al. demonstrated that neonephrogenesis could be induced in contralateral kidney by a two-third excision of one kidney in Leucoraja erinacea [3]. In contrast to lower animals, new nephron formation in mammals terminates...
shortly after birth. The postnatal mammalian kidney responds to partial resection by regenerative hypertrophy without neonephrogenesis. The response to partial resection of kidney during prenatal period is variable. There is evidence that new nephron formation can be induced during prenatal period by partial resection of kidney. For example, Douglas-Denton et al. showed a 45% increase in nephron number in contralateral kidney following unilateral nephrectomy in unborn sheep [4]. Neonephrogenesis of varying degrees seen in various organisms raises an important question: can kidney regeneration in mammals be facilitated by induction of neonephrogenesis following injury?

Kidneys are made up of nephrons that are surrounded by vasculature and connective tissue. There are more than 30 different cell types in an adult kidney, some of which are terminally differentiated, while others can proliferate following injury. Although resection of kidney is a rare clinical event, patients frequently present with kidney injury due to ischemia or toxins. In majority of patients, injured kidneys regenerate sufficiently to restore normal kidney function; however, in a significant number of patients, kidney regeneration is incomplete with grave consequences [5]. None of the available therapies can facilitate kidney regeneration, and patients with severe kidney injury are supported by dialysis for variable length of time while awaiting spontaneous regeneration. In addition, evidence in experimental animals show that even when kidney function returns to baseline, there is persistent residual damage at the cellular level that can progress over time to chronic kidney disease [6]. Therefore, therapeutic strategies for facilitating kidney regeneration are urgently required. Stem cell therapy is one such therapy that has provided hope for patients with kidney disease. In this manuscript, we have discussed the current state of knowledge in kidney regeneration based on stem cell biology.

Kidney Injury

There are two types of kidney injury: (a) Acute Kidney Injury (AKI) and (b) Chronic Kidney Disease (CKD). AKI is seen within days to weeks following the onset of acute injury and is reversible in a majority of patients. In AKI, there is cellular injury without damage to the connective tissue scaffold that supports cellular elements. With time, new cells are able to repopulate the existing scaffold and restore normal kidney function. In contrast, in CKD there is slow progressive damage to both cellular and non-cellular elements of the kidney. Therefore, replacing only the cellular elements of kidney in patients with CKD may not be sufficient for restoring normal kidney function. Accordingly, for ease of understanding, we have divided this manuscript into two sections: therapeutic use of stem cells for AKI and CKD.

Stem Cells and Acute Kidney Injury

Acute kidney injury results from damage to renal cells due to ischemia or toxins. In a majority of patients, an injured kidney is restored by replacement of damaged cells with new ones. New cells can arise from proliferation of existing mature surviving cells, stem cells present in the injured kidney, or from stem cells located outside the kidney. These three potential cellular sources of kidney regeneration are not mutually exclusive and can be requirement specific. Based on our present understanding of kidney regeneration, the role played by endogenous extra-renal stem cells appears to be minimal, and regeneration is believed to occur predominantly from phenotypically mature cells that have survived injury [7]. Although endogenous extra-renal stem cells do not contribute significantly to kidney regeneration, potential benefit of exogenously administered extra-renal stem cells should not be discarded prematurely. It has been shown both in animal models and patients that exogenously administered stem cells of non-renal origin can facilitate regeneration following acute kidney injury [8, 9].

Exogenously administered stem cells following acute kidney injury can improve outcome by the following three potential mechanisms: replacement of damaged cellular units with new ones which are the progeny of stem cells; supplementation of growth factors and cytokines by the administered stem cells that facilitates regeneration from the surviving mature kidney cells; and secretion of the extracellular matrix. Initially, cell per cell unit replacement by the progeny of stem cell was believed to play an important role; however, well-done studies have now shown that a non-cellular mechanism of kidney regeneration following exogenous administration of stem cells is the main mechanism of action. This mechanism of action due to a provision of growth factors and cytokines at the site of injury is called “paracrine mechanism” of action [10].
Of numerous stem cells that have been tested for treatment of acute kidney injury, mesenchymal stem cells (MSCs) are the most promising cell types for clinical use. We will not be discussing therapeutic use of other stem cells and refer readers to a recent review written on the topic [11]. In animal models, administered MSCs immediately reach injured kidneys; however, they are cleared within 24 hours [12]. This lead to the hypothesis that it is the non-cellular mechanism of action of MSCs – paracrine effect – that facilitates kidney regeneration. It is now known that MSCs secrete cytokines and growth factors that have protective effects in kidneys; many of these molecules have now been identified and partially characterized [10]. The paracrine mechanism of action of infused MSCs is further supported by experiments demonstrating the beneficial effect of MSC cell culture supernatant in facilitating kidney regeneration [13]. Promising results in animal models have resulted in Phase I clinical trial of use of MSCs in patients at high risk for developing AKI following cardiovascular surgery [14]. The results of this trial are very promising. This trial was performed to determine the safety and feasibility of such an approach in patients with acute kidney injury. Presently, investigators are testing the use of MSCs for treating AKI in a multicenter Phase II clinical trial. Although results of initial use of MSCs in human AKI are very promising, it will be premature to conclude safety and efficacy of such an approach in patients with acute kidney injury. Presently, investigators are testing the use of MSCs for treating AKI in a multicenter Phase II clinical trial. Although results of initial use of MSCs in human AKI are very promising, it will be premature to conclude safety and efficacy of such an approach in patients with acute kidney injury. Paracrine mechanisms of action of MSC raise an interesting possibility of identifying molecules that play important roles in kidney regeneration that can be developed into novel therapeutic agents.

Stem Cells and Chronic Kidney Disease

Presently, patients with advanced CKD are supported by dialysis for the rest of their life or until a new kidney is transplanted. Shortage of suitable organs for transplantation and complications of immunosuppression has led to a search for novel therapies for treating patients with CKD. Such novel therapies include renal assist device, refurbishing organ scaffolds with new cells, neonephrogenesis in bioartificial matrix, and use of embryonic kidneys for transplantation. Many of these approaches are still in the early stages of development; however, some have reached initial stages of clinical testing. Early clinical results and preclinical studies with bioartificial organs and tissue engineering are encouraging. These novel approaches offer some unique advantages and pose new scientific and technical challenges. For example, the membrane in a bioartificial organ protects donor cells from the patient immune system. Here we discuss such innovative therapies that may become available in the future for treating patients with CKD.

Renal Assist Device

The current day dialysis therapy substitutes only the filtration function of kidney. Besides filtration, the kidney performs other important functions such as reabsorption, metabolism, synthesis, and immunomodulation. Renal tubular cells perform these additional functions predominantly. In the renal assist device (RAD), the filtration function of present-day dialyzer is supplemented with a second hollow fiber dialyzer in series, which is lined by a monolayer of renal proximal tubular cells [15]. These cells form a confluent monolayer that performs important functions including vectorial transport, metabolic, synthetic, and immunomodulatory functions. Presently, cells needed for the renal assist device are isolated from the adult mammalian (porcine and human) kidneys. Potentially, cells needed for renal assist device can be obtained from stem cells that are differentiated to renal cells of desired phenotype. Stem cells are particularly attractive as a source of mature kidney cells as a large number of cells can be obtained from a relatively small number of initiating stem cells, and stem cells can be differentiated into multiple mature phenotypes, expanding the possibility of cell types that are substituted in the renal assist device.

In the Phase II multicenter, randomized, controlled, open-labelled trial, survival advantage of RAD over CRRT (continuous renal replacement therapy) was compared in 58 patients with AKI [16]. Although there was no survival advantage observed of RAD over CRRT at 28 days \( p = 0.08 \), it reached statistical significance at 180 days \( p = 0.038 \). The overall safety profile and stability of the device at 72 hours was acceptable in the Phase II trial. The concept and results of the initial use of RAD in AKI are encouraging and experiments have successfully matured the technology to a clinically applicable level using commercially available high-flux hemofiltration hollow fiber cartridges. If major limitations of the RAD therapy, such as availability...
of renal tubular cells in sufficient numbers and widespread availability of the device for clinical use can be achieved, RAD has the potential to become an important option in modern day therapy for patients with AKI and CKD.

**Refurbishing Organ Scaffolds with New Cells**

Frequently, donor kidneys are deemed unsuitable for transplantation because of damage to the cellular elements of the donor kidney. The scaffold made up of non-cellular matrix and connective tissue is often intact in such discarded donor kidneys. If such donor kidneys are seeded with fresh cells that are able to acquire mature phenotype, orientation, and function, refurbished kidneys may become suitable for transplantation. Use of natural organ scaffolds provides several advantages over synthetic matrix such as organ-specific micro-architecture, ideal adhesion surface and molecules, and required extracellular signals for proper differentiation. The proof of principle experiment validating the use of organ scaffold for generating refurbished organ was first shown in the heart [17]. Subsequently, Ross et al. have shown that kidney scaffolds can also be successfully cellularized using ES cells. Undifferentiated ES cells following homing into the decellularized kidney scaffold progressively expanded into vasculature and tubules and expressed kidney specific markers [18]. Presently, this technology is in its infancy; however, it is not out of realm that in the future, it can be translated into clinical practice.

**Nephron Formation in a Synthetic Matrix**

Although kidney scaffolds provide the best milieu for differentiating stem cells to mature renal cells, a biosynthetic matrix has also been successfully used for neonephrogenesis. In an intriguing study, Lanza et al. showed metanephric stem cells derived from Holstein steer can form new nephrons when placed in a synthetic matrix and transplanted subcutaneously [19]. Furthermore, new nephrons spontaneously connected with the draining tubes designed in the synthetic matrix and excreted fluid that was higher in waste products than the recipient animal’s blood, which is suggestive of active transport. Potentially this technology can evolve in the future and be used for augmenting nephron numbers in patients with chronic kidney disease. If this technology matures sufficiently, it opens numerous possibilities such as implanting biosynthetic nephrons under kidney capsule that can connect spontaneously with the existing drainage system of native kidneys similar to new nephrons formed in the biosynthetic matrix.

**Organs Derived from the Embryonic Kidney**

Interestingly, when a developing embryonic kidney is transplanted into an adult animal, it continues to grow, mature, develop vasculature of recipient origin, and form ureter that can be anastomosed with the urinary system and can contribute to renal function of the host. Presently, this technology is limited by the arrest of growth of transplanted embryonic kidneys beyond a certain developmental stage. Renal function contributed by a single embryonic kidney is minimal and insufficient for sustaining life of the recipient animal. Potentially, this limitation can be overcome by transplanting multiple embryonic kidneys; however, it will require transplantation of an impractically large number of embryonic kidneys to achieve meaningful life-sustaining function. Therefore, it is critical that this field evolves and embryonic kidneys can be matured further to provide meaningful kidney function on transplantation. Supply of human embryonic kidneys is an ethical issue. Interestingly, embryonic kidneys can be transplanted across the isogenic, allogenic, and xenogenic barriers due to the ability of embryonic tissue to induce tolerance [20-23]. Therefore, xenogenic kidneys can be transplanted into humans without the risk of rejection. Furthermore, Yokoo et al. have successfully partially humanized rat kidneys by injecting human mesenchymal stem cells expressing glial cell-derived neurotrophic factor (GDNF) interact embryos [24]. To summarize, transplantation of embryonic kidney is still in its infancy and has long ways to go before it can be clinically used. However, these proof of principle experiments clearly convey the scientific potential of this technology.

**Summary**

Kidney disease has reached epidemic proportions and is rapidly increasing. None of the current therapies facilitates kidney regeneration following injury. Majority of such injured kidneys spontaneously recover, while others remain permanently damaged and can progress onto chronic kidney disease. Stem cells have offered hope to such patients and many innovative stem cell based therapies are being
developed for patients with kidney disease. Such therapies include the administration of stem cells, refurbishment of kidney scaffolds with new cells, development of new nephrons in synthetic matrix, and transplantation of embryonic kidneys. Although most of these novel therapies are in early stages of development, use of mesenchymal stem cells for acute kidney injury has reached clinical trials. Other innovative stem cell-based therapies, which are in early stages of development, can also reach clinical use with evolution of technology and improved understanding of stem cell biology, and hold the promise of providing paradigm shifting treatment options for patients with kidney disease in the future.

Reference