Can patient-specific stem cell therapy enhance renal repair?*

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Stem cells

The advancement of stem cell biology has not only enhanced our understanding of organogenesis and tumourigenesis, but has also helped in designing stem cells as a therapeutic tool to accelerate tissue repair. Despite a complex array of genetic alterations needed for differentiation of pluripotent stem cells into a particular subset of cells, retroviral transduction of as little as three transcription factors, Oct3/4, Sox2 and Klf4, can transform adult cells into cells mimicking pluripotent embryonic stem cells [1]. The ability of reprogramming differentiated somatic cells towards pluripotency by the nuclear transfer method has paved the way to generate patient-specific stem cell therapy without ethical concerns. This article will briefly summarize the potentials of stem cell therapy to enhance renal repair.

Stem cells have the unique ability to differentiate and self-regenerate. A pleuripotent stem cell has the ability to differentiate in all the three germ layers, while the self-regenerating ability can increase the population of stem cells. Until very recently, research was directed towards identifying and characterizing three different types of stem cells (embryonic, bone marrow-derived and organ-bound) and exploring the prospects of using these cells to repair or regenerate damaged tissues and organs [2–7]. The necessity of artificial generation of stem cells came from the fact that the number of stem cells is much lower in adults than in embryos, partly because most stem cells in early embryonic life differentiate into specialized cell types to facilitate organogenesis. Moreover, isolating stem cells from adult tissues is difficult. Such limitations have motivated investigators to plan on using embryonic stem cells for potential therapeutics. However, embryonic stem cells have both therapeutic limitations (risk of rejection) and ethical concerns. A potential breakthrough in stem cell research has been achieved by the ability of reprogramming adult cells to those that closely resemble embryonic stem cells using four genes: Oct3/4, Sox2, Klf4 and c-Myc [1,8–10]; such an approach provides a unique clinical opportunity to use the adult cells obtained from a patient to genetically reprogramme the cells according to the particular need of the patient (or personalized medicine) (Figure 1). To reduce the potential risk of subsequent tumour formation in pluripotent stem cells, Nakagawa et al. have shown that by eliminating the protooncogene c-Myc, adult cells can still be reprogrammed to pluripotent cells by using the remaining three factors (Oct3/4, Sox2 and Klf4) [1]. Furthermore, of 36 mice injected with pluripotent stem cells generated through retroviral transduction of four genes, 6 mice died from tumours after 100 days, while out of 36 mice injected with pluripotent stem cells that produced with the three genes (without c-Myc), none of the mice died [1]. Needless to say, 100 days is a relatively short time for cells to be deemed harmless; however, the ability to exclude a protooncogene is an important step towards finding safer reprogrammed stem cells.

Despite the enormous potential of using adult cell-derived stem cell therapy [8,10], it should be mentioned that reprogramming of adult cells to pluripotent stem cells is based on virus-mediated transfer of genetic information [1,8–10]. Such virus-mediated transfer carries a risk of contamination and is a potential health hazard. Further studies are needed to find an alternative approach to end the virus-mediated method of reprogramming adult cells to become pluripotent stem cells in order to achieve efficient clinical benefits.

Artificial generation of stem cells

Embryonic stem cells can derive from the embryoblast, the inner cell layer of the blastocyst. The outer cell layer of the blastocyst is the trophoblast, which develops into the placenta. Embryonic stem cells, derived from blastocyst, can differentiate, in vivo, into cells of endoderm, mesoderm and ectoderm, and subsequently mature into organ-specific cells. In-depth understanding of embryonic development and organogenesis has inspired researchers to explore the
Fig. 1. Reprogramming of adult dermal fibroblasts to cells that closely resemble embryonic stem cells can be achieved by retroviral transduction of the Oct3/4, Sox2, Klf4 and c-Myc transcription factors [8]. Such reprogrammed cells can be therapeutically used as a tool to induce and enhance tissue regeneration and repair.

potential use of stem cells as a therapeutic tool to repair and regenerate adult tissues following injury.

The knowledge gained from somatic-cell nuclear transfer to clone the sheep, Dolly, was employed to clone several other animal species [11–13]. It is worth mentioning that the cloning of the dog Snuppy from the dog Tai by somatic-cell nuclear transfer, as claimed by the Korean scientists [13], was validated and the authenticity of the claim was confirmed by an independent laboratory work [14]. A slightly modified approach has been employed for humans, where a patient’s DNA was injected into an enucleated unfertilized egg to generate stem cells; these cells were cultured and allowed to differentiate in vitro, and then transplanted back into the patient. Such therapeutic cloning may minimize immune-mediated rejection of transplanted stem cells. It is necessary to mention that reprogramming somatic cells to stem cells by the fusion of somatic cells results in pluripotent cells that contain two sets of chromosomes and thereby limit the clinical use of generated stem cells [15,16]. Recently, scientists were able to reprogramme mouse somatic cells towards pluripotency by retroviral transduction of four transcription factors: Oct3/4, Sox2, Klf4 and c-Myc; these cells were also able to form embryoid bodies, teratomas and tissues originating from all three germ layers in chimeric mouse embryos [9]. Some of these observations may form the preliminary basis of generating personalized stem cell therapy without ethical concerns of utilization of mammalian unfertilized oocytes. Such possibilities are substantiated by the fact that, similar to mouse cells, human adult cells can be transformed into cells mimicking pluripotent cells by delivering defined genetic information [8].

Another intense area of research is to determine whether transplanted stem cells can recreate various essential compartments of the kidney. Due to the structural complexity of the kidney where resident cells need complex cellular interactions to produce urine, any attempt to artificially grow or repair a kidney will need to have distinct structures including glomeruli, tubules, interstitium and vessels. Lanza et al. recently explored such possibilities; by a nuclear transplantation technique dermal fibroblasts of an adult cow were transferred into the perivitelline space of bovine oocytes and the resulting nuclear transfer embryos nonsurgically transferred into progestin-synchronized recipients. After 56 days, renal cells from metanephric were isolated and expanded in culture and then seeded on a specialized polymer tube, followed by implantation into the same cow from which the cells were cloned. Histological analysis of the explants revealed a well-developed renal structure comprising organized glomeruli-like, tubular-like and vascular elements; more importantly, cloned metanephric cells could produce a urine-like liquid, suggesting the ability of artificially generated stem cells to grow into kidney tissue [17].

**Stem cells of the kidney: systemic versus resident**

Since the kidney has the ability to regenerate following various injuries, studies are focusing on the potential use of stem cell therapy in accelerating such regenerative processes, and thereby delaying the occurrence of end-stage renal failure. Once the sources of stem cells in the kidney are clearly defined, approaches to reactivate quiescent renal stem cells or facilitate the systemic supply of stem cells in the kidney may help in renal repair following injury. Research in recent years has identified the presence of both bone marrow-derived stem cells and quiescent stem cells residing in the kidney.

Bone marrow stem cells can differentiate into various renal cells including mesangial cells [18], tubular epithelial cells [19] and podocytes [20]. Moreover, bone marrow stem cell abnormalities have been shown to affect renal function, raising the possibility of the existence of a bone–kidney stem cell axis [21,22]. This possibility is further substantiated by the observation of Y chromosome-positive tubular epithelial cells in the transplanted kidney of a male patient who received a kidney transplant from a female donor [20]. In general, bone marrow-derived stem cells can migrate towards a site of injury and differentiate under the appropriate microenvironment [23]. The circulating precursor cells can not only transdifferentiate, but can also fuse with the neighbouring cells to repair damaged tissue [24]. The interaction of CD44 and its ligand hyaluronic acid has been shown to influence the exogenous mesenchymal stem cells to localize in the kidneys with experimentally induced acute renal failure to enhance renal repair [25]. The extent and involvement of the bone marrow-derived stem cells in renal repair is, however, an unsolved issue, and an intense area of research.

In addition to systemic stem cells, another possibility that has gained research interest is the search for quiescent stem cells migrating and populating in the kidney during development. CD133, a general surface marker of tissue stem cells [26,27], has been detected in the human adult kidney [28], and when CD133-positive human kidney cells were injected into the SCID mice, these cells could differentiate to form renal tissue. Along a similar line of study, stem cell antigen-1 (Sca-1)-positive cells of mouse renal interstitium...
can differentiate into tubular epithelial cells when injected directly into the kidney following ischaemia/reperfusion injury [29]. Similarly, a subset of parietal epithelial cells in the Bowman’s capsule coexpress stem cell markers CD24, CD133 and stem cell–specific transcription factors Oct4 and Bmi1 in adult human kidneys. Both CD24- and CD133-positive parietal epithelial cells have self-renewal potential and a high cloning efficiency, in vitro. In the proper microenvironment, individual clones of CD24- and CD133-positive parietal epithelial cells could generate renal tubular epithelial cells, in addition to osteogenic cells, adipocytes and neuronal cells. Furthermore, transfer of CD24- and CD133-positive parietal epithelial cells into SCID mice with acute renal failure could help in tubular regeneration, and could ameliorate the morphologic and functional kidney damage [30]. It thus appears that both systemic and resident renal stem cells can differentiate into mature renal cells, and thereby can be used as a therapeutic tool to enhance renal repair.

How may stem cell therapy impact renal medicine?

Maintaining water, electrolyte and mineral balance and eliminating metabolic waste are the main functions of the kidney [31,32]. Most of the chronic renal diseases, without therapeutic intervention, usually progress to irreversible renal failure [33–35] to affect water, electrolyte and mineral balance. Since one of the major functions of adult stem cells or somatic stem cells is to replace or repair damaged tissues or organs, studies are focused on using stem cells for renal repair. Recent studies have suggested potential roles of both haematopoietic and intrinsic renal stem cells in the repair process following kidney injury [36,37]. For instance, Lin et al. reported the potential role of bone marrow stem cells in regeneration of renal proximal tubular cells following renal ischaemia/reperfusion injury in mice [37]. In a similar study, Kale et al. also showed the benefits of bone marrow stem cell infusion in ischaemia/reperfusion injury in mice [38], suggesting a therapeutic application of exogenous stem cells to repair renal injury. The potential of mesenchymal stem cell–based therapies is an interesting option as these cells can be obtained from adult individuals; such possibility is further substantiated by the observation of the ability of mesenchymal stem cells to repair damaged tissues in experimental renal diseases [39–42]. In a similar line of study [36], using chimeric mice in which the mature renal tubular epithelial cells and their progeny were permanently labelled with EGFP to determine the role of intrinsic versus exogenous cells during renal repair after ischaemic/reperfusion injury, 89% of proliferating epithelial cells were shown to originate from the host cells, and the remaining 11% were shown to originate from the donor bone marrow cells [36]. It appears that bone marrow–derived cells might have a relatively minor role in renal repair. In a separate study, Stokman et al. could not ameliorate renal fibrosis by enhanced mobilization of bone marrow–derived cells in an experimental unilateral ureter obstruction model [43]. Likewise, haematopoietic stem cell mobilization–associated granulocytosis has been shown to severely damage renal structure and function in mice with ischaemia/reperfusion injury [44]. Furthermore, studies have also raised the question of specificity of the detection system of various tags that are usually used to track bone marrow–derived cells in the kidney. Various tags including LacZ, EGFP or a genetic marker (Y chromosome) were used to detect bone marrow–derived cells in the kidney; a recent study [45], however, demonstrated the possibility of false positive results in all the detection systems, suggesting the need for careful consideration of the contribution of bone marrow–derived cells in renal repair. Despite limitations and ethical concerns, stem cell therapy has the potential to redefine regenerative medicine and provide the hope of patient–specific renal repair in diseases where currently available therapies are ineffective [46].

Concluding remarks

Recent studies have focused on the therapeutic application of three types of stem cells: embryonic stem cells, bone marrow–derived stem cells and organ–bound stem cells. A fourth type of genetically reprogrammed adult cells that mimic endogenous stem cells has widened the therapeutic options. Theoretically, stem cells of different origins have the ability of unrestricted replication and, if adequately differentiated in the correct microenvironment, can become fully mature and functionally active renal cells. However, a more detailed understanding of the molecular and cellular events during differentiation of stem cells into a particular type of renal cell is required to use stem cells as a therapeutic tool to accelerate renal repair, and thereby delay progression of disease. Since six key genes, Lim1, Pax2, WTI, GDNF, c-Ret and Wnt4 are essential for kidney development, it will be of interest to determine whether adult stem cell differentiation needs certain specific factors to generate a particular type of renal cells [47–50]. Moreover, studies have shown that Wnt4-transformed mouse embryonic stem cells can differentiate into renal tubular epithelial cells [51]. In addition, renotropic factors, including hepatocyte growth factor (HGF), epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-I), bone morphogenetic protein-7 (BMP-7) and leukaemia inhibitory factor (LIF) are required for branching morphogenesis of the ureteric bud and epithelial differentiation of metanephric mesenchymal cells during kidney development. Studies have shown that providing some of these renotropic factors can enhance tubular regeneration following renal injury [52–54]. Available studies suggest that renal stem cells, in the proper microenvironment, can differentiate into mature renal cells to repair renal injury. Both resident renal (major contributor) and systemic (minor contributor) stem cells may participate in such regenerative process. Stem cell therapy, therefore, has enormous potential in renal regenerative medicine and can be used to change the course of renal diseases to delay the progression of end-stage kidney failure.

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