

Stem Cell and Kidney Disease Treatment

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Abstract: Embryonic stem cells, pluripotent derivatives of the inner cell mass of the blastocyst, are the most primitive cell type likely to find application in cell therapy. Their potential to generate any cell type of the embryo makes them to be the most attractive stem cell therapy. End-stage renal disease is a big health problem in the United States and in all places of the world. It is possible to introduce stem cells into a damaged adult kidney to aid in repair and regeneration. Transdifferentiation offers the possibility of avoiding complications from immunogenicity of introduced cells by obtaining the more easily accessible stem cells of another tissue type from the patient undergoing treatment, expanding them in vitro, and reintroducing them as a therapeutic agent. Adult stem cells may possess a considerable degree of plasticity in the differentiation. Immunoisolation of heterologous cells by encapsulation creates opportunities for their safe use as a component of implanted or ex vivo devices.

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1. Introduction

Stem cell is the origin of an organism's life. Stem cells have the potential to develop into many different types of cells in life bodies, which are exciting to scientists because of their potential to develop into many different cells, tissues and organs (Ma, 2005). End-stage renal disease (ESRD) is a big health problem in the United States and it costs more than \$30 billion each year on ESRD therapy in this country (Arnold, 2000; Mai et al., 2006; Ross et al., 2006). The patients suffering from acute renal failure (ARF) are even worse. ARF develops predominantly due to the injury and necrosis of renal proximal tubule cells (RPTCs) as a result of ischemic or toxic insult. The cause of death subsequent to ARF is generally the development of systemic inflammatory response syndrome. The disease state arising from renal failure is the result of many factors. It is important to reveal the kidney's role in reclamation of metabolic substrates, synthesis of glutathione, free-radical scavenging enzymes, gluconeogenesis, ammoniogenesis, hormones, growth factors, and the production and regulation of multiple cytokines critical to inflammation and immunological. There is considerable drive to develop improved therapies for renal failure (Mehta et al., 2007). It is estimated that there are over 2 million patients in USA who suffer from end-stage renal disease. About 60,000 patients in the United States are currently on the waiting list for a kidney transplant, and some patients have waited for several years before an appropriate donor can be found. Despite the advances in kidney transplantation, the shortage of donor organs limits treatment for the

ESRD patients and requires many patients to remain on dialysis for extended periods of life time. The alternate methods has resulted in rapid progression of new approaches, such as therapeutic cloning and stem cell therapy (Berzoff et al., 2008; Sirmon, 1990).

Some of the most notable findings are as follows: (1) the 'stemness' profile may be determined by approximately 250 genes; (2) organ-specific stem-cell growth and differentiation are stimulated during the reparative phase following transient injury; (3) two bone marrow stem-cell types show a remarkable degree of differentiation potential; (4) some organs contain resident marrow-derived stem cells, and their differentiation potential may only be expressed during repair; (5) the metanephric mesenchyme contains pluripotent and self-renewing stem cells; (6) marrow-derived cells invade the kidney and differentiate into mesangial and tubular epithelial cells, and these processes are increased following renal injury; and (7) epithelial-to-mesenchymal transition generates renal fibroblasts (Oliver, 2004).

Cell therapy is dependent on cell and tissue culture methodologies to expand specific cells to replace important differentiated functions lost or deranged in various disease states. Cell-based therapeutics is the question of cell sourcing, and advance of stem cell research is powerful on the resolving of this problem. Stem cell is at present a great deal of speculation over the extent to which stem cell populations traditionally considered distinct may in fact be interchangeable. Stem cells can be self-renewal to differentiate into specialized cell types and they are classified as totipotent, pluripotent and multipotent.

Progenitor cells are more lineage-restricted than stem cells but retain the proliferative capacity lacking in terminally differentiated cells (Lin et al., 1996).

Embryonic stem (ES) cells, pluripotent derivatives of the inner cell mass of the blastocyst, are the most primitive cell type likely to find application in cell therapy. Their potential to generate any cell type of the embryo makes them to be the most attractive stem cell therapy. However, the political and ethical debates surrounding the use of human ES cells make the application of stem cells complicated. These factors have combined to intensify the focus on. Neural stem cells (NSCs) are self-renewing, multipotent cells that generate the neuronal and glial cells of the nervous system. In mammals, contrary to long-held belief, neurogenesis occurs in the adult brain, and NSCs reside in the adult central nervous system (Taupin, 2006; Winkler, 2003).

It is possible to introduce stem cells into a damaged adult kidney to aid in repair and regeneration. Transdifferentiation offers the possibility of avoiding complications from immunogenicity of introduced cells by obtaining the more easily accessible stem cells of another tissue type from the patient undergoing treatment, expanding them *in vitro*, and reintroducing them as a therapeutic agent. Adult stem cells may possess a considerable degree of plasticity in the differentiation. However, the differentiation of stem cells is normally unresolved. Pluripotent cells can be derived from fibroblasts by ectopic expression of defined transcription factors. A fundamental unresolved question is whether terminally differentiated cells can be reprogrammed to pluripotency (Hanna et al., 2008).

Developing nephrons are derived from renal stem cells and transplantation of fetal kidneys may be thought of as a therapeutic stem cell application. There are two bioengineering programs with the aim of producing a device providing full renal replacement therapy in the short to medium term. Both employ biomaterial scaffold structures to overcome the as yet insurmountable difficulties inherent in marshalling cells into organized three-dimensional structures capable of coordinated filtration, resorption/metabolism/secretion, collection, and disposal of waste. Initial experiments involved adult rabbit renal cortex harvested and fractionated into glomeruli, distal, and proximal tubules, expanded separately *in vitro*, and seeded onto biodegradable polyglycolic acid sheets for subcutaneous implantation into syngenic hosts. The potential impact of advances in stem cell technology on all the prospective cell-based therapeutic approaches for the treatment of renal failure discussed above is enormous. The kidney has a dramatic capacity

to regenerate after injury. Whether stem cells are the source of the epithelial progenitors replacing injured and dying tubular epithelium is an area of intense investigation. Many surviving renal epithelial cells after injury become dedifferentiated and take on mesenchymal characteristics. These cells proliferate to restore the integrity of the denuded basement membrane, and subsequently redifferentiate into a functional epithelium. An alternative possibility is that a minority of surviving intratubular cells possess stem cell properties and selectively proliferate after damage to neighboring cells. Some evidence exists to support this hypothesis but it has not yet been rigorously evaluated (Vigneau et al., 2007).

In recent years, it has been shown that functional stem cells exist in the adult bone marrow, and they can contribute to renal remodelling or reconstitution of injured renal glomeruli, especially mesangial cells, and hMSC found in renal glomeruli differentiated into mesangial cells *in vivo* after glomerular injury occurred (Wong et al., 2008).

2. Isolation of Kidney Stem Cells

It is difficult to find a definitive marker for kidney stem cells that makes it difficult to isolate and define kidney stem cells. However, kidney stem cells have been isolated from other organs using 4 different ways. For the first method, when the DNA of the cells is labeled with a marker such as bromodeoxyuridine, the cells retain the label for a long period of time. This label retention is used to identify and isolate stem cells. The second method references the side-population (SP) cells that extrude Hoechst dye through the activity of multidrug resistance proteins, which are part of the ATP-binding cassette transporter superfamily. The third method isolates kidney stem cells referencing specific cell surface markers that have been used to identify stem cells in other organs or the metanephric kidney. The markers used to isolate kidney stem cells include Oct-4, Nanog, CD24, CD133 and stem cell antigen-1 (Sca-1). The fourth method uses culture conditions that select stem cells in other organ systems (<http://content.karger.com/produktedb/produkte.asp?typ=fulltext&file=000117311#SA4>).

As Zheng et al described in 2008, any unique characteristic can be used to isolate a pure population of stem cell is still lacking. There is few specific biomarker found for epidermal stem cells alone, but epidermal stem cells and transient-amplifying cells share some biomarkers (Bickenbach, 2003) (Zheng, et al, 2008).

A brief protocol for stem cell culture is shown in Figure 1.

Brief protocol for stem cell culture

1. Trypsinize cells in the exponential phase of growth (varies for each cell type, but typically is after 3 days of growth). First aspirate the medium and wash the culture twice with 3 mL of PBS per 35-mm dish and then add 1 mL of trypsin/EDTA. Incubate under the phase contrast microscope.
1. After about 3 min cells begin to round with clearly defined edges.
2. Once cell rounding is observed, add 3 mL of medium with serum and pipet several times to disaggregate cells from the dish and from each other until a single cell suspension is achieved.
3. This is a general trypsinization procedure. The medium added after trypsinization should be the same as the cells are currently cultured in.
4. Pellet the cells by centrifugation ($200 \times g$, 4–5 min, at room temperature) and resuspend in an appropriate amount of cell culture medium.
5. For convenience, cells are frozen in 1-ml aliquots at cell numbers that correspond to the appropriate numbers that will be needed upon thawing. The upper limit would be $5-10 \times 10^6$ cells/ml.
6. 4. Slowly add an equal volume of the freezing medium drop-wise over 2 min. Continuously shake the cell suspension for even distribution of the DMSO/FBS freezing medium.
7. 5. Aliquot 1-ml of cell suspension into cryovials.
8. ESCs are typically frozen between 5×10^5 and 1×10^6 cells/mL.
9. 6. Immediately transfer cryovials to a Cryo 1°C Freezing Container and place the container in a -70°C or a -80°C freezer for 24 hr.
10. 7. Transfer the vials to liquid nitrogen.

Figure 1. A brief protocol for stem cell culture

A possible design for animal usage in a stem cell study is shown in Table 1.

Table 1. Mice used in the project

Mouse groups (total n=300)	Treatment and purpose
Group 1, mating, female, n=40	Normal mice, mating for pregnant to get ES cells
Group 2, mating, male, n=40	Normal mice, mating with female for ES cell
Group 3, sham, male, n=20	Normal mice, as sham
Group 4, control, male, n=100	Obstructed mice, control, no stem cell treatment
Group 5, stem cell treated, male, n=100	Obstructed mice, treated with stem cell

Total animals used: 300.

3. Culture of Renal Stem Cell

Shimony et al characterized a new model of renal, stromal and mesenchymal stem cell (MSC) matrix deprivation, based on slow rotation cell culture conditions (ROCK). This model induces anoikis using a low shear stress, laminar flow. The mechanism of cell death was determined via FACS (fluorescence-activated cell sorting) analysis for annexin V and propidium iodide uptake and via DNA laddering. Their results showed while only renal epithelial cells progressively died in STCK, the ROCK model could induce apoptosis in stromal and transformed cells; cell survival decreased in ROCK versus STCK to 40%, 52%, 62% and 7% in human fibroblast, rat MSC, renal cell carcinoma (RCC) and human melanoma cell lines, respectively. Furthermore, while ROCK induced primarily apoptosis in renal epithelial cells, necrosis was more prevalent in transformed and cancer cells [necrosis/apoptosis ratio of 72.7% in CaKi-1 RCC

cells versus 4.3% in MDCK (Madin-Darby canine kidney) cells]. The ROCK-mediated shift to necrosis in RCC cells was further accentuated 3.4-fold by H₂O₂-mediated oxidative stress while in adherent HK-2 renal epithelial cells, oxidative stress enhanced apoptosis. ROCK conditions could also unveil a similar pattern in the LZ100 rat MSC line where in ROCK 44% less apoptosis was observed versus STCK and 45% less apoptosis versus monolayer conditions. Apoptosis in response to oxidative stress was also attenuated in the rat MSC line in ROCK, thereby highlighting rat MSC transformation. They concluded that the ROCK matrix-deficiency cell culture model may provide a valuable insight into the mechanism of renal and MSC cell death in response to matrix deprivation (Shimony et al., 2008)

Celprogen Company has human kidney stem cell derived from human adult and fetal kidney

consented tissue. The following is the information and technique of the kidney stem cells:

(1) Positive markers:

CD34, Nestin & CD133.

(2) Morphology and proliferation:

Mixed population of cells with approximately 70% attached cells and the other 30% in suspension; need to change cell culture media every day after 48 hours of initial cell culture or when the media starts changing color to slight yellow for pink. Fast growing cell culture. Change media with Celprogen's Human Kidney Stem Cell Complete Growth Media with the appropriate Human Stem Cell Extracellular matrix and tissue culture media for differentiation, expansion or maintaining stem cells in their un-differentiated stage. Temperature 37°C in 5% CO₂ humidified incubator.

(3) Subculturing:

- A. Thaw the vial with gentle agitation in a 37°C water bath or a dry 37°C shaking incubator. For water bath thawing
- B. keep the O-ring out of the water, thawing time 2-3 minutes.
- C. Remove the thawed vial and wipe with 70% ethanol. Then transfer to the tissue culture hood.
- D. Transfer the vial contents to a 15 ml sterile centrifuge tube, and gently add 7ml of pre-warmed Human Kidney Stem Cell Complete Media to the centrifuge tube. Use an additional 0.5 ml of Human Kidney Stem Cell complete media to rinse the vial and transfer the liquid to the centrifuge tube repeat this once more to ensure you have all the cells transferred to the 15 ml centrifuge tube. Add 1 ml of Human Kidney Stem Complete Media to bring the final volume to 10 ml in the 15 ml centrifuge tube.
- E. Centrifuge the cells at 100 g for 5 minutes. Remove the supernatant and resuspend the cell pellet in 500 ul of Human Kidney Stem Cell Complete Growth Media.
- F. Add the 500 ul of cells to T75 flask pre-coated with Human Kidney Stem Cell Extracellular matrix with 15 ml of Human Stem Cell Complete Growth Medium.
- G. Incubate the cells in the T75 flask in a 37°C in 5% CO₂ humidified incubator. Perform 100% Media Change every 24 to 48 hours.
- H. Medium renewal every day, and recommended sub-culturing ratio: 1:3.

(4) Freezing Medium:

Human Stem Cell Complete growth Medium supplemented with 90% Fetal Bovine Serum with 10% DMSO.

(5) Storage temperature:

Liquid nitrogen vapor phase (San Pedro, CA 90731, USA, <http://www.celprogen.com>; http://ftp.celprogen.com/Technical_Resources/36100-27%20Human%20Kidney%20Stem%20cell%20data%20sheet.pdf).

In mice with cisplatin-induced acute kidney injury, administration of bone marrow-derived mesenchymal stem cells (MSC) restores renal tubular structure and improves renal function (Imberti et al., 2007).

4. Application of Kidney Stem Cells

Stem cell has powerful potential application purpose in science, medicine and industry, but it is also potentially danger for its unexpected plasticity. The evidence for bone marrow-derived stem cell contributions to renal repair has been challenged. The research and application for adult renal stem cells are also debated. The use of embryonic tissue in research continues to provide valuable insights but will be the subject of intense societal scrutiny and debate before it reaches the stage of clinical application. Embryonic stem cells, with their ability to generate all of kind of cell in living body, are great chance for our human civilization but have ethical and political hurdles for human use (Brodie and Humes, 2005). Stem cell research has attracted great attention because it could be used for the regeneration of damaged organs that are untreatable by conventional medical techniques, and stem cells (such as endothelial stem cells and neural stem cells) have been discovered to be practical useful in clinical applications. The potential for stem cell gene therapy has increased and many therapeutic applications have already been done. Chronic renal failure is a candidate for stem cell gene therapy. In the application of renal stem cell in medical treatment, mesenchymal stem cells could be transplanted, and in contrast, hematopoietic stem cells may be used for gene delivery for diseases, which need foreign cytokines and growth factors, such as glomerulonephritis. The stem cell gene therapy for chronic renal failure and the potential of the novel strategy and the major practical challenges of its clinical application are big targets for the stem cell researches (Yokoo et al., 2003). Ectopic expression of the human telomerase reverse transcriptase gene in human mesenchymal stem cells can reconstitute their telomerase activity and extend their replicative life-spans (Li, et al, 2007).

Discussion

Kidney is derived from the ureteric bud and metanephrogenic mesenchyme, and these two progenitor cells differentiate into more than 26 different cell types in 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, indicating that the kidney contains epithelial stem cells. These stem cells also are present in the adult kidney. Embryonic renal epithelial stem cells can generate other cell types (Al-Awqati and Oliver, 2002). The key important target in kidney stem cell research and application is to get kidney stem cells from other types of the cells, and it is also important to find the better way to change kidney stem cells to other cell types.

As the nature will, to live eternally is an extracting dream in all the human history. Stem cell is the original of life and all cells come from stem cells. Germline stem cell (GSC) is the cell in the earliest of the cell stage. It is possible to inject the GSC into adult human body to get the eternal life. This article is to try to describe the stem cell and to explore the possibility of the eternal life with the stem cell strategy. The production of functional male gametes is dependent on the continuous activity of germline stem cells. The availability of a transplantation assay system to unequivocally identify male germline stem cells has allowed their in vitro culture, cryopreservation, and genetic modification. Moreover, the system has enabled the identification of conditions and factors involved in stem cell self-renewal, the foundation of spermatogenesis, and the production of spermatozoa. The increased knowledge about these cells is also of great potential practical value, for example, for the possible cryopreservation of stem cells from boys undergoing treatment for cancer to safeguard their germ line (Ma, et al, 2007).

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