Stem Cell and Granulocyte Colony-stimulating Factor (GCSF)

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Abstract: The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell". Stem Cell is the original of life. All cells come from stem cells. Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situation or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, a bone cell, a nerve cell, or a brain cell. Stem cell research is a typical and important topic of life science. This material collects some literatures on stem cell and granulocyte colony-stimulating factor (GCSF) and stem cell.
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Introduction

Chronic kidney disease is increasing at the rate of 6-8% per year in the Unite States. At present, dialysis and transplantation are the common treatment options. However, it is possible to use stem cells and regenerative medicine as the additional choices for kidney disease treatment. Such new treatments might involve induction of repair using endogenous or exogenous stem cells or the reprogramming of the organ to reinitiate development (Hopkins et al. 2009).

In the 20th century, an efficient treatment was given to patients with renal failure through the development of kidney dialysis and transplantation. These techniques have been proved successful, but are marred by inflammation and limited organ availability and graft survival due to immune rejection. More recently, hope has been placed in the development of stem cell-based therapies, in which the function of the failing organs is restored by injected multipotent cells. Possible sources for these cells include differentiated embryonic stem (ES) cells, adult renal stem cells, and circulating multipotent cells, such as bone marrow-derived stem cells. Using the patient's own stem cells to repair kidney damage could circumvent the problems of immune rejection and organ availability.

Kidneys can regenerate, which varies among species. Some bony and cartilaginous fish continue to form new nephrons during adult life. Adult mammals cannot form new nephrons but, to a certain extent, tubules and glomeruli may recover structure and function after limited injury such as acute tubular necrosis. Severe or prolonged injury results in replacement of functional parenchyma by scar tissue, i.e. fibrosis, which correlates clinically with the development of renal failure.

An effective treatment of renal disease is renal cell regeneration, or replacement of damaged renal cells, that discourage fibrosis. The origins for renal parenchymal cells could be: (1) the re-entry into cell cycle of differentiated cells; (2) direct transdifferentiation of one cell type into another, such as tubular cells into interstitial cells or vice versa; (3) differentiation from stem cells of the kidney or the bone marrow.

Embryonic stem cells are different from adult or tissue-specific stem cells. Embryonic stem cells are the stem cell that can be grown in large numbers in the laboratory and retain the ability to grow into any type of cells including renal, nerve, heart muscle, bone and insulin-producing cells. It is difficult for the tissue-specific adult stem cells to grow in a great number, hard to isolate and are difficult to grow outside the body. Adult stem cells, such as skin and bone marrow stem cells, normally grow into a limited number of cell types (Snykers et al. 2008).

The role of embryonic or adult stem cells, in particular bone marrow-derived stem cells, in regenerating the kidney after injury has been the subject of intensive investigation. Bone marrow-derived stem cells have been shown to give rise to small numbers of most renal cell types, including tubular cells, mesangial cells, podocytes, vascular cells and interstitial cells. Injections of bone marrow-derived cells do improve renal function in many animal models of renal disease. Many stages of nephrogenesis can be studied using cultured embryonic kidneys, but there is no efficient technique available to readily knockdown or overexpress transgenes for rapid evaluation of resulting phenotypes. Embryonic stem cells have unlimited developmental potential and can be manipulated at the molecular genetic level by a variety of methods. ES cell technology may achieve the objective of obtaining a versatile cell culture system in which

molecular interventions can be used in vitro and consequences of these perturbations on the normal kidney development program in vivo can be studied (Steenhard et al. 2005).

Stem cells and progenitor cells are necessary for repair and regeneration of injured renal tissue. Infiltrating or resident stem cells can contribute to the replacement of lost or damaged tissue. However, the regulation of circulating progenitor cells is not well understood. Many factors influence the stem cell growth in damaged kidney. For example, low levels of erythropoietin induce mobilization and differentiation of endothelial progenitor cells and erythropoietin ameliorates tissue injury. Full regeneration of renal tissue demands the existence of stem cells and an adequate local milieu, a so-called stem cell niche. It was reported that in the regenerating zone of the shark kidney, stem cells exist that can be induced by loss of renal tissue to form new glomeruli. Stem cell may eventually contribute to novel therapies of the kidney disease (Perin et al. 2008).

Researchers used a rat model of chronic renal failure in which one kidney is excised so as to increase the load of the remaining kidney, thus causing a chronic deterioration that resembles the clinical situation of renal failure (Alexandre et al. 2008). In Alexandre's project, **t**he rats were divided into 4 groups: Group 1 were sham operated and both kidneys left in place; Group 2 had a kidney removed but were not administered cells; Group 3 were administered $2x10^6$ lineage negative bone marrow cells on day 15 after one of the kidneys was removed; Group 4 were administered $2x10^6$ lineage negative bone marrow cells on days 15, 30, and 45 after one of the kidneys was removed. They found: (1) Expression of inflammatory cytokines was reduced on day 16 in the kidneys of rats receiving stem cells as compared to rats that were nephrectomized but did not receive cells. (2) On day 60 rats receiving stem cells had decreased proteinuria, glomerulosclerosis, anemia, renal infiltration of immune cells and protein expression of monocyte chemoattractant protein-1, as well as decreased interstitial area. (3) Injured rats had higher numbers of proliferating cells in the kidney, whereas rats receiving stem cells had less. (4) Protein expression of the cyclin-dependent kinase inhibitor p21 and of vascular endothelial growth factor increased after nephrectomy and decreased after stem cell treatment. (5) On day 120, renal function (inulin clearance) was improved in the rats which were administered bone marrow cells compared to controls. This study supports the possibility of using bone marrow cells for various aspects of kidney failure. Other studies have demonstrated that administered stem cells promote kidney repair by secretion of insulin growth factor-1 (Cornelissen et al. 2008).

Bone marrow stromal cells, also known as mesenchymal stem cells or fibroblastic colony-forming units, are multipotent non-hematopoietic stem cells adhering to culture plates (Abdallah and Kassem 2009). Mesenchymal stem cells of the bone marrow have the ability to renew and differentiate themselves into multiple lineages of conjunctive tissues, including bone, cartilage, adipose tissue, tendons, muscle, and bone marrow stroma. Those cells have been first described by Friedenstein et al., who found that mesenchymal stem cells adhere to culture plates, look like in vitro fibroblasts, and build up colonies (Friedenstein et al. 1987).

Bone marrow is the site of hematopoiesis and bone marrow transplant has been successfully used for decades as a means of treating various hematological malignancies in which the recipient hematopoietic compartment is replaced by donor-derived stem cells. Progenitor cells in bone marrow are capable to differentiate into other tissues, such as cardiac tissue. Clinical trials have been conducted demonstrating beneficial effects of bone marrow infusion in cardiac patients. It is believed that injured tissue, whether neural tissue after a stroke, or injured cardiac tissue, has the ability to selectively attract bone marrow stem cells, perhaps to induce regeneration. Bone marrow has therapeutic effect in conditions ranging from liver failure, to peripheral artery disease, and the possibility of using bone marrow stem cells in kidney failure has been relatively understudied (Ma et al. 2009).

Mesenchymal stem cells have been brought to the attention of many researchers, because these cells are of great interest for treating various human diseases. Many studies have isolated mesenchymal stem cells and controlled, in vitro, its differentiation into cartilaginous tissue and bone using specific growth factors, with the objective of using this technology for repairing injured tissues of mesenchymal origin (Xian and Foster 2006; Kurdi and Booz 2007).

Granulocyte colony-stimulating factor

Granulocyte colony-stimulating factor (GCSF) is a colony-stimulating factor hormone. GCSF is also known as colony-stimulating factor 3 (CSF 3). It is a glycoprotein, growth factor and cytokine produced by a number of different tissues to stimulate the bone marrow to produce granulocytes and stem cells. GCSF then stimulates the bone marrow to release them into the blood. GCSF also stimulates the survival, proliferation, differentiation, and function of neutrophil precursors and mature neutrophils. GCSF regulates them using Janus kinase

(JAK)/signal transducer and activator of transcription (STAT) and Ras/mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signal transduction pathway.

Mouse GCSF was first recognised and purified in Walter and Eliza Hall Institute, Australia in 1983, and the human form was cloned by groups from Japan and the Germany/United States in 1986.

GCSF is produced by endothelium, macrophages, and a number of other immune cells. The natural human glycoprotein exists in two forms, a 174- and 180-amino-acid-long protein of molecular weight 19,600 dalton. The more-abundant and more-active 174-amino acid form has been used in the development of pharmaceutical products by recombinant DNA (rDNA) technology.

The GCSF-receptor is present on precursor cells in the bone marrow, and, in response to stimulation by GCSF, initiates proliferation and differentiation into mature granulocytes. GCSF is also a potent inducer of HSCs mobilization from the bone marrow into the bloodstream, although it has been shown that it does not directly affect the hematopoietic progenitors that are mobilized.

Beside the effect on the hematopoietic system, GCSF can also act on neuronal cells as a neurotrophic factor. Indeed, its receptor is expressed by neurons in the brain and spinal cord. The action of GCSF in the central nervous system is to induce neurogenesis, to increase the neuroplasticity and to counteract apoptosis. These properties are currently under investigations for the development of treatments of neurological diseases such as cerebral ischemia.

The gene for GCSF is located on chromosome 17, locus q11.2-q12. Nagata et al. found that the GCSF gene has 4 introns, and that 2 different polypeptides are synthesized from the same gene by differential splicing of mRNA.

The 2 polypeptides differ by the presence or absence of 3 amino acids. Expression studies indicate that both have authentic GCSF activity.

It is thought that stability of the GCSF mRNA is regulated by an RNA element called the GCSF factor stem-loop destabilising element.

GCSF stimulates the production of white blood cells (WBC). In oncology and hematology, a recombinant form of GCSF is used with certain cancer patients to accelerate recovery from neutropenia after chemotherapy, allowing higher-intensity treatment regimens. Chemotherapy can cause myelosuppression and unacceptably low levels of white blood cells, making patients prone to infections and sepsis. GCSF is also used to increase the number of hematopoietic stem cells in the blood of the donor before collection by leukapheresis for use in hematopoietic stem cell transplantation. It may also be given to the receiver, to compensate for conditioning regimens.

Itescu planned in 2004 to use GCSF to treat heart degeneration by injecting it into the blood-stream, plus stromal cell-derived factor (SDF) directly to the heart.

It was first marketed by Amgen with the brand name Neupogen. Several bio-generic versions are now also available in markets such as Europe and Australia.

The recombinant human GCSF synthesised in an *E. coli* expression system is called filgrastim. The structure of filgrastim differs slightly from the structure of the natural glycoprotein. Most published studies have used filgrastim. Filgrastim (Neupogen) and PEG-filgrastim (Neulasta) are two commercially-available forms of rhGCSF (recombinant human GCSF). The PEG (polyethylene glycol) form has a much longer half-life, reducing the necessity of daily injections.

Another form of recombinant human GCSF called lenograstim is synthesised in Chinese Hamster Ovary cells (CHO cells). As this is a mammalian cell expression system, lenograstim is indistinguishable from the 174-amino acid natural human GCSF. No clinical or therapeutic consequences of the differences between filgrastim and lenograstim have yet been identified, but there are no formal comparative studies.

Filgrastim is a granulocyte colony-stimulating factor (GCSF) analog used to stimulate the proliferation and differentiation of granulocytes. It is produced by recombinant DNA technology. The gene for human granulocyte colony-stimulating factor is inserted into the genetic material of *Escherichia coli*. The GCSF then produced by *E. coli* is only slightly different from GCSF naturally made in humans.

It is marketed by Amgen under the brand name Neupogen, Dr. Reddy's Laboratories under the brand name Grafeel, Reliance Life Sciences under the brand name Religrast, Zenotech Laboratories Limited under the brand name Nugraf, Raichem Life Sciences under the brand name Shilgrast, Intas Biopharmaceuticals under the brand name Neukine and Emcure biopharmaceuticals under the brand name Emgrast.

Apricus Biosciences is currently developing and testing a product (under the brand name Nupen) which can deliver filgrastim through the skin to improve post-chemotherapy recovery of neutrophil counts.

Filgrastim is used to treat neutropenia (a low number of neutrophils), stimulating the bone marrow to increase production of neutrophils. Causes of neutropenia include chemotherapy and bone marrow transplantation. Filgrastim is also used to increase the number of hematopoietic stem cells in the blood

before collection by leukapheresis for use in hematopoietic stem cell transplantation. It is produced by many companies worldwide.

The kidney has the ability to regenerate various injuries, and the potential use of stem cell therapy in accelerating such regenerative processes, thereby delaying the occurrence of end-stage renal failure. Research in recent years has identified the presence of both bone marrow-derived stem cells and quiescent stem cells residing in the kidney [Nephrol. Dial. Transplant. (2008) 23 (6): 1826-1830] (http://ndt.oxfordjournals.org/content/23/6/1826.full).

Bone marrow stem cells can differentiate into various renal cells including mesangial cells (Imasawa, et al., 2001), tubular epithelial cells (Gupta, et al, 2002) and podocytes (Poulsom, 2001). 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 (Imasawa, et al., 1999; Terrier, et al., 2006). 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. In general, bone marrow-derived stem cells can migrate towards a site of injury and differentiate under the appropriate microenvironment (Zhang, et al, 2004). The circulating precursor cells can not only transdifferentiate, but can also fuse with the neighbouring cells to repair damaged tissue (Ying, et al., 2002). 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 (Herrera, et al., 2007). 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.

Embryonic Stem Cells (ES cells)

ES cells are pluripotent cells derived from the inner cell mass of blastocysts, and are in theory able to give rise to all the cell types of the body. ES cells can be directed into forming renal progenitor cells, and eventually differentiated renal cells. Ureteric bud epithelial cells and metanephric mesenchymal cells that comprise the metanephric kidney primordium are capable of producing nephrons and collecting ducts through reciprocal inductive interaction. Once these cells are induced from pluripotent ES cells, they have the potential to become powerful tools in regeneration of kidney tissues. However, there is **a** risk to use stem cells in clinical practice. In vivo, injection of ES cells can give rise to teratomas, which are tumors containing cells of all three lineages (ectoderm, endoderm and mesoderm). ES cell-derived teratomas in vivo, renal primordial structures, can be detected histochemically. Genes involved in metanephrogenesis express the potential of ES cells to produce renal primordial duct structures and provide the insight into the regeneration of kidney tissues (Yamamoto et al. 2006). This same potential was reported when ES cells were injected into embryonic mouse kidneys in vitro, and gave rise to ES cell-derived tubules, in this case without forming teratomas (Steenhard et al. 2005). In vitro, transfection of murine ES cells with renal developmental gene Wnt4, as well as the addition of hepatocyte growth factor and activin-A, both promote the formation of renal tubule-like structures, with expression of tubular marker aquaporin-2. Cultured Wnt4-EBs have an ability to differentiate into renal tubular cells; and second, that Wnt4, HGF, and activin A may promote the differentiation of ES cells to renal tubular cells (Kobayashi et al. 2005). The Wnt4-transfected cells can be transplanted into mouse renal cortex, where they also express aquaporin-2 and formed tubular structures. According to Kim et al reported, murine ES cells primed in vitro with retinoic acid, activin-A and BMP-7 (Kim and Dressler 2005), activin-A alone (Vigneau et al. 2007), or BMP-4, differentiate into cells expressing markers of the intermediate mesoderm, early kidney development and/or renal tubule-specific markers (Bruce et al. 2007). After injection of these primed murine ES cells into embryonic kidney cultures, ES cells are incorporated into developing renal tubules (without cell fusion) or into the nephrogenic zone. The primed cells are enriched for renal progenitor cells by FACS and are injected in vivo into the kidneys of newborn mice, where they are integrated as proximal tubular cells, without teratoma formation (Vigneau et al. 2007). Human ES cells differentiate in vitro into WT1- and renin-expressing cells following treatment with a combination of specific growth factors (Schuldiner et al. 2000). However, research of the role for ES cells in renal regeneration is still in its infancy (Roufosse and Cook 2008).

Native Renal Stem Cells and Renal Regeneration

In the embryo, most types of renal parenchymal cells are derived from metanephric mesenchymal cells, which are multipotent and are in addition self-renewing, making them attractive candidates as the stem cells of the embryonic kidney.

In animal models, embryonic metanephroi transplanted into the abdominal cavity of adult animals are colonized by host vasculature, undergo nephrogenesis and produce urine, even if the operation is carried out across species barriers, and with a surprising lack of rejection (Little 2006). Human and porcine embryonic kidney progenitor cells

have been isolated and, when injected into mice, can lead to the formation of miniature kidneys producing urine (Dekel et al. 2003), or protect against acute renal failure (Lazzeri et al. 2007). However, there are ethical issues to deal with human ES cells.

In adult mammals, a range of methods have been used to identify potential multipotent precursor cells, including label retention in slow cycling cells, identification of a side population, and expression of stem cell markers such as CD133. This has led to the identification of several candidate renal stem cells, which, depending on the study, are located amongst the tubular cell population (Dekel et al. 2006; Gupta et al. 2006), in the Bowman's capsule, papillary region or cortical interstitium (Bussolati et al. 2005; Sagrinati et al. 2006; Rad et al. 2008). Of note, other studies have not confirmed the presence of a large pool of precursor cells amongst the tubular population and, instead, argue that regeneration occurs through proliferation of differentiated tubular cells (Vogetseder et al. 2008; Witzgall 2008). Some of the candidate renal stem cells have been shown to enhance recovery after tubular injury, possibly by integration in the tubular epithelium (Rad et al. 2008).

Bone Marrow-Derived Stem Cells and Renal Regeneration

Bone marrow stem cells would be an ideal source of multipotent cells: they are easy to harvest and are in theory an unlimited source of expandable autologous cells. They display an unexpected plasticity which has been the subject of extensive research over the last few years. The plasticity has been observed both for the hematopoietic stem cell, which gives rise to all differentiated blood cell types, as well as for the bone marrow mesenchymal stem cells, which provide stromal support for haematopoietic stem cell in the bone marrow, and also give rise to various mesenchymal tissues, such as bone, cartilage and fat.

There are important discrepancies in the literature addressing the role of bone marrow cells in renal regeneration. The technique most commonly used to study bone marrow cell plasticity is bone marrow transplantation. The host bone marrow is replaced by donor bone marrow, and after bone marrow chimerism is established, donor cells are tracked down in the kidney. The donor bone marrow cells are distinguished from host cells by virtue of their chromosome content (male Y chromosome-positive cells in a female host), the expression of a reporter molecule (β -galactosidase, luciferase, enhanced green fluorescent protein), or the performance of a function (re-establishment of a function in a knockout mouse model). The type of host cell that the bone marrow-derived cell has given rise to (tubular, mesangial, etc.) is ascertained most often using immunohistochemistry.

Discrepancies between studies are attributable to several factors: (1) observations in different species (mouse, rat, human); (2) use of different models of renal damage (ischaemia/reperfusion, toxic, immunological); (3) different protocols for bone marrow transplantation (irradiation doses, quantity of cells injected); (4) injection of different subgroups of bone marrow cells (whole bone marrow, haematopoietic stem cell, mesenchymal stem cell); (5) sensitivity and specificity of the detection method for bone marrow cell origin (in situ hybridization for the Y chromosome, detection of reporter molecules, functional assays), and (6) sensitivity and specificity of the detection method of the renal cell type (immunohistochemistry for specific cell types such as tubular cell, mesangial cells, etc.).

Renal failure can be the result of an initial insult directed against the tubular epithelium, the glomerular cells or the vascular compartment. In the search for remedies for these varied renal diseases, studies have therefore addressed potential bone marrow origin for various renal cell types. It is useful to bear in mind these technical variations when analysing results reported in the literature (Roufosse and Cook 2008).

Tubular Epithelium

Although initial studies suggested a high contribution of bone marrow to tubular regeneration, the current view is that only a small proportion of tubular cells are bone marrow-derived, and there is disagreement over whether mesenchymal stem cells, haematopoietic stem cells or both are contributing (Humphreys and Bonventre 2008). The current consensus view is that the predominant source of tubular regeneration is through the proliferation of differentiated tubular cells (Lin et al. 2005). A few authors have not found any bone marrow cells engrafted in tubules, and propose that positive observations of bone marrow-derived tubular cells are the result of artifact (Bussolati et al. 2009). There may also be a progenitor slow-cycling cell population contributing to tubular repair.

Firstly, under certain circumstances, bone marrow engraftment in tubules can be dramatically increased. Held et al. made use of a transgenic fumarylacetoacetate (FAH)-/- mouse, in which discontinuation of the rescue drug NTBC leads to acute tubular necrosis (Held et al. 2006). After transplanting bone marrow from wild-type mice into FAH-/- mice, a few bone marrow-derived tubular cells are noted. In a subset of the FAH-/- mice, there is, in addition, loss of heterozygosity (LOH) in the liver for homogentistic acid hydrogenase, which induces a

more severe, ongoing form of acute tubular necrosis. In FAH-/- animals with additional hepatic LOH, up to 50% of tubular cells are bone marrow-derived cells. Engraftment of these wild-type bone marrow-derived cells leads to morphological resolution of ATN and to disappearance of the aminoaciduria present in control mice. In this model, the bone marrow cells have a strong survival advantage over native tubular cells, due to their ability to metabolise toxic products. It is possible that this strong positive selective pressure is necessary for regeneration to occur through wild-type bone marrow cells. Interestingly, most of the bone marrow-derived tubular cells are derived from cell fusion between bone marrow cells and tubular cells. This is supported by a study by Li et al. in which fusion of bone marrow cells to tubular cells account for part of bone marrow-derived tubular cells after ischaemia/reperfusion (I/R) injury, but not all. In this model without selective pressure, the percentage of bone marrow-derived tubular cells is low (1.8%) (Li et al. 2007b).

Secondly, although there is disagreement concerning the underlying mechanism, injection of bone marrow cells, particularly mesenchymal stem cells, has repeatedly been shown to improve renal function in ATN, whether induced by toxins (cisplatin and glycerol) or I/R (Imai and Iwatani 2007). With the role of actual engraftment of bone marrow cells as tubular cells thought to be minimal or absent, mesenchymal stem cells may exert their beneficial effects through their antiapoptotic, mitogenic, immunomodulatory and angiogenic properties, or through the contribution of the bone marrow cells to endothelial cell replacement in the peritubular capillaries. It is important to know the nature of the mediators involved in these properties, and the mechanisms governing the homing of mesenchymal stem cells to the kidney (Imai and Iwatani 2007). Imberti et al. confirmed the importance of paracrine mechanisms using co-culture of mesenchymal stem cells with tubular cells in a Transwell® culture excluding contact between the two cell types, which led to less cisplatin-induced tubular cell death. mesenchymal stem cells have been shown to produce vascular endothelial growth factor, basic fibroblast growth factor, monocyte chemoattractant protein-1, hepatocyte growth factor, and insulin-like growth factor, as well as immunomodulators TGF-β and PGE_2 (Imai and Iwatani 2007; Imberti et al. 2007). In a recent study, administration of conditioned medium from cultured stromal cells provided the same renoprotective effects as injection of mesenchymal stem cells, suggesting that systemic administration of the beneficial mediators may be just as good as mesenchymal stem cell injection, and safer (Imberti et al. 2007). It is a concern that there have been a few observations of adipogenesis associated with fibrosis and osteogenesis after injection of mesenchymal stem cells (Imai and Iwatani 2007).

Mesenchymal stem cell homing to the kidney has been linked to interactions between molecules upregulated in the injured kidney (SDF-1, hyaluronic acid and PDGF) and ligands expressed on mesenchymal stem cells (respectively, CXCR4, CD44 and PDGF-R) (Imai and Iwatani 2007). Similar beneficial effects on renal function may be induced by mobilizing bone marrow cells from the patient's own bone marrow by administration of growth factors (GF) such as granulocyte colony-forming factor, granulocyte/monocyte colony-forming factor, monocyte colony-forming factor, and stem cell factor. Possible explanations for improved renal function include increased numbers of bone marrow-derived tubular cells, a decrease in neutrophilic infiltrate, or increased cell proliferation and decreased apoptosis in kidneys of GF-treated mice (Roufosse and Cook 2008).

In summary, most but not all authors agree that a small proportion of tubular cells (at most a few percent) are bone marrow-derived after renal injury. The role these bone marrow-derived tubular cells play in improved renal function is probably insignificant, with intrinsic renal cells, either stem cells or differentiated, more likely to play the predominant role in regeneration. However, administration of bone marrow cells or mobilization of bone marrow cells using GF may be used to protect against renal injury. This may be due to paracrine / immunomodulatory effects or endothelial regeneration. In addition, there may be a therapeutic role for bone marrow-derived cells engineered to replace a defective gene, due to a local strong positive selective pressure. mesenchymal stem cells have emerged as the most promising candidate for stem cell therapy, and appear safe, such that phase I clinical trials of mesenchymal stem cell injection for the treatment of acute kidney injury are scheduled to begin shortly (Imai and Iwatani 2007).

Mesangial Cells

Mesangial cells are modified smooth muscle cells in the glomerular tuft, and provide structural support for the complex of glomerular capillaries. They may be injured by immune complex deposition, toxins and in diabetes. Although mesangial cells have regenerative potential, persistent mesangial damage can lead to glomerulosclerosis. In cell culture, bone marrow cells treated with PDGF-BB in the presence of collagen IV convert to cells with many mesangial characteristics (Suzuki et al. 2004). In rodent models of bone marrow transplantation, there is also support for partial bone marrow derivation of mesangial cells, whether glomeruli are injured or not. In models where

mesangial damage has been induced, infusion of bone marrow cells may be associated with improved function, which has been attributed to mesangial and endothelial regeneration or, in the case of mesenchymal stem cells, paracrine mechanisms. Conversely, a deleterious mesangial phenotype responsible for mesangial sclerosis, such as in Os/- or db/db mice, can be induced by transplanting wild-type mice with transgenic mouse bone marrow. Some studies have further illustrated functionality of the bone marrow-derived mesangial cells by harvesting the bone marrow-derived mesangial cells, growing them in culture, and showing angiotensin-II induced contraction in vitro, a typical mesangial function (Kunter et al. 2006).

Podocytes

Podocytes are epithelial cells with complex interdigitating foot processes which create the slit diaphragm, and contribute to the synthesis of the glomerular basement membrane. Both the slit diaphragm and the glomerular basement membrane are implicated in creating a filtration barrier between blood and urine. Initial studies identified rare bone marrow-derived cells at the periphery of the glomerular tuft, in the location of podocytes. Two recent studies have suggested integration of bone marrow-derived cells as functional podocytes, with production of matrix protein. These studies involved the use of a mouse model of Alport's disease, in which the animals suffer from defective synthesis of the alpha-3 chain of collagen type IV, with glomerular basement membrane abnormalities and progression to glomerulosclerosis and renal failure. Using whole bone marrow transplantation from wild-type animals, both Prodromidi et al. and Sugimoto et al. showed the presence of bone marrow-derived podocytes and mesangial cells, accompanied by re-expression of the defective collagen chains, and improved renal histology and function. Although the bone marrow-derived cells were not numerous, their presence was sufficient to re-establish synthesis of the defective collagen chain. However, the improvement in renal function was substantial raising the possibility that there may be mechanisms involved other than replacement of podocytes. A similar experiment using mesenchymal stem cells only rather than whole bone marrow also led to a reduction in interstitial fibrosis, but without engraftment of bone marrow cells in the kidney, and with no beneficial effect on survival or renal function (Sugimoto et al. 2006).

Vascular Cells

Endothelial cells are present in the glomerular capillaries, in large vessels and in the abundant network of peritubular capillaries. Endothelial cells are attractive candidates for progeny of bone marrow-derived cells in view of their immediate contact with circulating cells, the existence of known circulating endothelial precursors, and the existence of a known endothelial precursor in the bone marrow: the haemangioblast.

In a rat model of glomerulonephritis, where glomerular endothelial cells are injured, culture-modified bone marrow mononuclear cells injected into the renal artery boosted renal regeneration. This was attributed both to incorporation of bone marrow-derived cells into the endothelial lining and to production of angiogenic factors by the injected cells. Similarly, following acute tubular necrosis, the peritubular capillaries are damaged. The return of blood flow, which depends on endothelial cell integrity, is essential for renal recovery. Duffield et al. contend that bone marrow cells boost renal function after I/R by participating in endothelial cell regeneration. Li et al. observed bone marrow-derived vWF+ and CD31+ endothelial cells in a mouse model of adriamycin-induced nephrosis with subsequent renal fibrosis (Li et al. 2007a).

Interstitial Cells

The kidney contains a complex population of interstitial cells serving several functions, such as providing a scaffold for renal structure and producing several hormonal substances such as erythropoietin. It may even contain a population of adult native renal stem cells which play a role in renal regeneration. There is also evidence that bone marrow-derived cells could be a source for up to 30% of α-SMA-positive interstitial myofibroblasts, which have been incriminated in the production of extracellular matrix in renal fibrosis. If the bone marrow is indeed a source for such cells, the use of bone marrow cell injections for the treatment of renal failure would run the risk of enhancing fibrosis (Broekema et al. 2007).

Adipose Stem Cells

Adipose, also known as fat tissue, is the richest and most accessible known source of stem cells. It contains a specialized class of stem cells comprised of multiple cell types that promote healing and repair. Adipose stem cells have been shown to differentiate into multiple cell types including muscle, bone, fat, cartilage and nerve, etc. Beyond differentiation, regenerative cells may provide therapeutic benefit through the release of growth factors and other therapeutic healing mechanisms. The major advantages of adipose tissue as a source of regenerative cells, which distinguish it from alternative cell sources, include: **(1) Yield:** A therapeutic dose of regenerative cells can be isolated in approximately one hour without cell culture. **(2)**

Safety: Patients receive their own cells (autologous-use) so there is no risk of immune rejection or transmission. **(3) Versatility:** Stem cells from adipose tissue benefit from multiple mechanisms-of-action.

There is crucial disagreement on the issue of functionality of these cells. Do the bone marrow-derived cells actively participate in extracellular matrix synthesis? Roufosse et al. in a mouse model of unilateral ureteric obstruction, detected bone marrow-derived α-SMA-positive cells. Using two reporter molecules under the control of the promoter and enhancer elements of the collagen I α2 chain gene, we did not however observe any functional bone marrow-derived fibroblasts or myofibroblasts producing collagen I. On the other hand, Iwano et al. in a mouse model of unilateral ureteric obstruction, and Broekema et al. in a rat model of unilateral I/R injury, demonstrated double immunostaining positivity of α-SMA-positive interstitial cells with pro-collagen I protein (Broekema et al. 2007).

In this project, with the mouse model, we aim to find the practical conditions to induce ES cells differentiating into renal stem cells and to find the ways using the renal stem cells to repair and regenerate obstructed kidney. For these, we will explore the techniques to induce ES cells to adopt a renal fate using co-culture with cell lines, metanephroi and novel growth factors. Meantime, we will characterize the expression profile of different renal subcompartments so as to identify the secreted proteins involved in renal differentiation and to isolate the specific cell surface markers identifying renal stem cells.

The theoretical background justifying the pursuit of the potential of bone marrow cells to participate in renal regeneration has been laid. Stem cells, both embryonic and from the adult bone marrow, in the right conditions, can express renal markers in vitro and give rise to renal cells in vivo.

In addition, injection of stem cells into the kidney or the bloodstream can lead to an improvement of renal function, although this does not always seem to be mediated by transdifferentiation into renal cells. Current views favour a predominant role for the delivery of a cocktail of angiogenic and immunomodulatory mediators as the main means by which bone marrow cells enhance epithelial and endothelial cell survival. As far as engraftment of bone marrow cells as renal parenchymal cells is concerned, proving functionality of the engrafted bone marrow-derived cells is crucial in order to assign to them a role in improved renal function, rather than relying on morphological observations alone.

The kidney is a complex organ with over 30 different cell types, and present technology does not envisage constructing a whole kidney from stem cells. However, within existing kidneys where the basic scaffolding is intact, stem cells may contribute to a variety of specialised cell types, either promoting more efficient repair or correcting genetic defects. These would include: (1) acute tubular necrosis (ATN) caused by toxins or ischaemia/reperfusion (associated with kidney transplantation); (2) mesangial damage, often associated with immune complex deposition and diabetes; (3) defective podocyte function (Alport's disease); (4) vascular endothelial damage (e.g. in glomerulonephritis) (Alison et al. 2007; Alison 2009).

Whether stem cell injections will ever be used for the treatment of renal failure is at this stage still unknown. There is certainly some hope to be found in the numerous animal models that have been developed and analysed over the last few years.

To reach the goal, we propose the following basic research objectives: (1) Use expression profiling to further dissect the processes of commitment to a metanephric fate during normal development. (2) Identify novel renal progenitor cell markers and growth factors to assist in the identification, isolation and/or reactivation of renal stem cells. (3) Examine the potential for ES cells to be differentiated into the lineages necessary for renal regeneration or endogenous repair.

4. Research Design and Methods

4.1 GCSF gene primer design and cDNA synthesis

A. Construction of GCSF

cDNA is produced by reverse transcriptase-mediated polymerase chain reaction (RT-PCR) using total RNAs isolated from human monocytes. A modified hGCSF cDNA (mhGCSF) lacking signal sequence, G+C content at 5' coding region of which is reduced without altering the predicted amino acids sequence and the codon TGC (C17A) is replaced with codon GCC, is produced by PCR by three steps based on the template as described above. These alterations respectively facilitate its translation in E coli and inhibit the formation of disulfide-linked oligomers to stabilize hGCSF. In first step, 5' 55 bp fragment is made by forward primer 5' CAT ATG ACA CCC CTA GGC CCT GCC 3' and reverse primer 5'C TTG CTC TAA **GGC** CTT GAG CAG G3'. 3' 484 bp fragment is made by forward primer 5'C TTA GAG CAA GTG3' and reverse primer 5'TGAATTCA TTA GGG CTG GGC AAG GT A3'. Both of fragments are denatured, annealed and elongated with DNA polymerase at 96°C for 10 minutes and at 72°C for 20 minutes. Finally, the modified 540 bp GCSF (mhGCSF) is made based with the following: CAT ATG ACA CCC CTA GGC CCT GCC as forward; primer; and TGAAT TCA TTA GGG CTG GGC AAG GT as reverse primer (Figure 1). The amplified

products are fractionated on agarose gels. The GCSF cDNA eluted from the agarose gel is digested by Bam HI and EcoR I, and ligated to pBluescript II SK that has been cut with the same restriction enzymes. The ligation mixture is transformed into Escherichia coli DH5α competent cells for characterization of the cloned cDNA, and is confirmed by a thorough DNA sequencing analysis.

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