

Review Article

Adult stem cells in renal injury and repair

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SUMMARY: There has been considerable focus on the ability of bone marrow-derived cells to differentiate into non-haematopoietic cells of various tissue lineages, including cells of the kidney. This growing evidence has led to a reconsideration of the source of cells contributing to renal repair following injury. The kidney has an inherent ability for recovery and regeneration following acute damage. It is thought that dedifferentiation of glomerular and tubular cells to a more embryonic/mesenchymal phenotype represent key processes for recovery in response to damage. However, there has been much contention as to the source of regenerating renal cells. The present review focuses on new aspects of the plasticity of intrinsic renal cells and their role in renal remodelling and scarring. Growing support also suggests that bone marrow-derived cells have the ability to contribute to structural and functional repair following acute renal failure. Evidence for bone marrow cell engraftment in the repairing kidney leading to incorporation into a variety of tissue types is discussed. Because cell death and fibrosis is a common end-point in a variety of acute and chronic renal nephropathies, the paradigm of stem cell plasticity may have important implications in the cellular and pathological mechanisms of renal injury and repair. A better understanding of the processes controlling extra-renal cell engraftment and intrinsic renal cell differentiation may provide important clues for the development of new cell-based therapies in the field of renal reparative medicine.

KEY WORDS: bone marrow stem cells, cell fusion, EMT, renal regeneration, reparative medicine, transdifferentiation.

INTRODUCTION

Given the considerable morbidity from long-term dialysis treatment and the ever increasing organ transplant waiting lists, there is clearly a need for new renal replacement therapies. The most common final pathway for patients with chronic renal disease is cell loss, accumulation of extracellular matrix (ECM) proteins, and the development of interstitial fibrosis. Many studies have focused on the cellular and molecular events leading to the development of renal interstitial fibrosis, but less is known about mechanisms promoting cellular repair and tissue remodelling. Several recent clinical and experimental studies suggest that circulating adult haematopoietic and mesenchymal stem cells (MSC) show considerable plasticity. Bone marrow stem cells are traditionally considered to give rise to blood cells (hae-

matopoietic stem cells; HSC) and connective and adipose tissue (MSC), but there is evidence that they may also be capable of generating an array of cell lineages that contribute to various tissues and organs. In the present review, evidence for renal cell plasticity and the emerging concept of transdifferentiation and cell fusion playing important roles in the recovery of kidney after injury will be explored. Current strategies emerging from the diverse field of renal reparative medicine will also be discussed.

ADULT VERSUS EMBRYONIC STEM CELLS

Stem cells are characterized by their capacity for long-term self-renewal, and the ability to differentiate into specialized tissue types. They are found in adult and embryonic tissues and have potential uses in therapies designed to repair and regenerate organs.

Embryonic stem (ES) cells are derived from the inner cell mass of the blastocyst and are termed 'pluripotent' because they have the potential to form any tissue type in the body.^{1,2} The pluripotent nature of ES cells may pro-

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vide many potential therapeutic applications. The use of ES cells for transplantation depends on the ability to push differentiation toward a required tissue type and the prevention of uncontrolled growth of inappropriate tissue types.³ Delivery techniques will also need to be developed to introduce *in vitro* differentiated ES cells to target organs. Rejection complications may arise if a host's immune system recognizes transplanted ES cells as being non-self. The use of human embryos for the production of ES cells has raised many ethical issues that remain unresolved.

Adult stem cells exist in various tissue-specific guises and have been reported in organs such as bone marrow,⁴⁻⁶ brain,⁷ the peripheral nervous system,⁸ heart,⁹ skeletal muscle⁶ and skin.¹⁰ The normal differentiation repertoire of adult stem cells is more limited than that of ES cells and they typically give rise to one or more cell types found in the organ in which they reside. Adult stem cells are responsible for tissue repair and renewal, processes that it is hoped can be harnessed for therapeutic use. Further understanding of how to encourage the activity of resident and circulating stem cells could form the basis of future reparative therapies that take advantage of existing adult stem cell differentiation and trafficking mechanisms. Alternatively, preparations of harvested adult stem cells could be used in transplantation-based therapies similar to those proposed for ES cells. An advantage of using adult stem cells is that they could be harvested from the individual being treated to prevent the graft rejection problems that occur when non-self tissue is recognized by a recipient's immune system. Adult stem cells are also unlikely to give rise to the uncontrolled growth of inappropriate tissue types that occurs when undifferentiated ES produce tumours (teratomas) following transplantation.

BONE MARROW STEM CELL PLASTICITY AND ORGAN REPAIR

Recent research has suggested that the plasticity of adult stem cell may be broader than initially thought. Under certain conditions, adult stem cells have been reported to cross what were previously thought to be 'lineage boundaries' to produce unexpected cell types. The majority of these reports of adult stem cell plasticity have involved HSC. Haematopoietic stem cells are a well-studied class of adult stem cell that localize predominantly to the bone marrow and are responsible for production of the cell types of which blood is composed. The differentiation potential of HSC may not be limited to blood and various studies have reported bone marrow-derived cells in the kidney, as well as other organs such as the heart,^{11,12} liver, lung, gastrointestinal tract, skin,¹³ brain^{14,15} and blood vessels.¹⁶ These findings suggest that HSC have the ability to differentiate into organ cell types that were previously considered to be outside their haematopoietic

lineage boundary. Many of the aforementioned studies have reported that injury promotes the incorporation of bone marrow-derived cells into organs. This observation strengthens the argument that the incorporation of bone marrow-derived cells is part of an organ repair process. Mesenchymal stem cells are another class of bone marrow-localized stem cell that has the ability to generate bone, cartilage and fat. Mesenchymal stem cells have also been reported to demonstrate unexpected plasticity and incorporation into various organs.¹⁷ It must be noted that the mechanisms, low frequency rates and significance of the incorporation of bone marrow-derived cells into organs are controversial topics that are only beginning to be rigorously investigated.¹⁸

CONTRIBUTION OF ADULT STEM CELLS IN RENAL REPAIR

Increasing evidence suggests that bone marrow-derived cells can be induced to replace specialized adult renal cells in humans and animal models. Studies of this phenomenon have used human patients or experimental animals that have received a kidney or bone marrow transplant. These transplantation procedures have resulted in individuals with genetically dissimilar kidney and bone marrow tissue and has allowed the identification of bone marrow-derived cells in the kidney.

Bone marrow cells have been observed to contribute to kidney repair in a clinical setting. These studies have relied on tracing the male-specific Y chromosome in male patients receiving a kidney transplant from a female donor, or a female patient receiving bone marrow from a male donor. Bone marrow-derived cells have been reported in the renal vasculature and interstitium,¹⁹ renal tubules²⁰⁻²² and the glomerulus.²¹ Immunostaining of Y chromosome-containing bone-marrow derived cells in the renal tubule and glomerulus has shown that they are negative for markers that would suggest that they are simply infiltrating white blood cells.²⁰⁻²² Furthermore, bone marrow-derived cells in the renal tubule are positive for epithelial markers, suggesting that they have adopted a tubular phenotype.²⁰⁻²²

Clinical findings are supported by several animal studies reporting bone marrow-derived cells in the kidney. Poulosom *et al.* studied kidneys of female mice that received a male bone marrow transplant following irradiation.²¹ Approximately 8% of tubular epithelial cells contained a Y chromosome, with a smaller number that had the physical appearance of podocytes observed in the glomerulus.

In other experimental animal models, bone marrow cells have been reported to differentiate into proximal tubular epithelial cells, mesangial cells, endothelial cells, and interstitial cells. Transient ischaemic injury of the kidney has been used as an experimental model to determine the ability of bone marrow cells to contribute to the

repopulation of damaged renal cells. Lin *et al.* transplanted a purified population of haematopoietic stem cells from Rosa26 mice that constitutively express β -galactosidase, into non-transgenic recipient female mice.²³ Following ischaemia–reperfusion injury, the donor-derived cells evident with X-gal staining were detected primarily in the S3 segments of proximal tubules. This finding was supported by polymerase chain reaction (PCR) detection of the male-specific Sry gene and fluorescence *in situ* hybridization (FISH) detection of the Y chromosome in the kidneys of female transplant recipients. Approximately 80% of X-gal-positive tubules contained cells that co-expressed proximal tubular markers but a Y chromosome was detected in only 8% of tubular cells.²³ No evidence of bone marrow cell engraftment was observed in distal tubules or collecting ducts, cells less susceptible to ischaemic insult. In control animals undergoing bone marrow transplantation without ischaemia–reperfusion injury there was no evidence of engraftment, suggesting that integration of bone marrow-derived cells is dependent on an inflammatory environment. Likewise, Kale *et al.* transplanted sorted Lin[−]Sca-1⁺c-kit⁺ bone marrow cells from Rosa26 mice into irradiated recipient mice and demonstrated that they homed to regions of ischaemic injury.²⁴ In this study, approximately 20% of tubules in the outer medulla were β -galactosidase-positive and were also found to express the proximal tubular marker megalin. Morigi *et al.* also demonstrated that the administration of purified MSC immediately following tubular injury results in stem cell-derived tubular epithelial cells and an improvement in kidney function.²⁵

Recently, Masuya *et al.* reported that a single haematopoietic cell was capable of differentiating into mesangial cells in lethally irradiated recipient mice.²⁶ Haematopoietic chimerism evident in glomerular mesangial cells was observed 2 months after transplantation. This study supports previous evidence suggesting that bone marrow cells normally reconstitute mesangial cells and interstitium.^{27,28} Imasawa *et al.* reported that green fluorescent protein (GFP)-positive bone marrow cells, localized to glomeruli and interstitium of irradiated mice, are important in the normal turnover of renal cells.²⁷ In glomeruli of chimeric rats, bone marrow-derived endothelial cells and mesangial cells have been observed in anti-Thy-1.1-glomerulonephritis.²⁹ Using immunofluorescent double-labelling with renal specific markers, the engrafted bone marrow cells were found to remain after restoration of glomerular architecture. These studies suggest that renal repair may not only involve recovery by proliferation of resident cells, but also recruitment of bone marrow-derived vascular progenitor cells.²⁹

The ability of bone marrow-derived cells to contribute to kidney tissues explains reports that renal disease can be transmitted by bone marrow transplantation. The phenotypes caused by genetic defects leading to glomerulosclerosis and diabetic nephropathy have both been

reported to be transmissible by bone marrow transplantation in mice.^{30,31}

RENAL CELL TRANSDIFFERENTIATION VERSUS FUSION

Transdifferentiation and epithelial–mesenchymal transition of renal cells

The process of transdifferentiation, describing the phenotypic conversion of one cell to another cell type, is one mechanism by which stem cells could possibly contribute to the repair of tissue arising from different lineages. This observed pluripotent nature of adult cells leads us to re-evaluate the concept of transdifferentiation that is evident in intrinsic renal cells during injury and remodelling.

The pathogenesis of renal interstitial fibrosis involves the infiltration of inflammatory cells, proximal tubular injury and death, ECM accumulation, and the progressive loss of renal function. Fundamentally linked to the development of interstitial fibrosis is the transformation of interstitial fibroblasts to α -smooth muscle actin (SMA) + ve myofibroblasts.^{32,33} During this process, both glomerular and tubular cells have been shown to change phenotype during the course of remodelling.^{34–36} This observed cell plasticity has been termed ‘epithelial to mesenchymal transition’ (EMT), a process wherein glomerular and tubular cells lose their epithelial phenotype and undergo mesenchymal transformation.^{37,38} During renal development mesenchymal cells differentiate into epithelial cells, and likewise, during a fibrotic response epithelial cells revert to a more mesenchymal state before undergoing repair. This process of EMT, that has also been termed ‘reverse embryogenesis’, may be a link between nephrogenesis and cell differentiation of the adult kidney in order to facilitate the course of healing.^{34,39}

The plasticity of epithelial cells undergoing EMT during injury is an essential process in the course of repair (Fig. 1). The extent of tubular repair and the role of EMT in the subsequent development of interstitial fibrosis are dependent on the degree of injury. The EMT of tubular cells is associated with hypertrophy, loss of brush border, and a rapid change in cell polarity. Depending on the extent of injury, epithelial cells either elongate and undergo EMT, or eventually detach and undergo cell death.³⁵ The integrity of the basement membrane, expression of integrins and growth factors, and synthesis of type IV collagen are all important factors for successful cell repair and reattachment.⁴⁰

Interstitial fibroblasts also play an essential role in kidney fibrosis and repair by EMT-dependent mechanisms in response to growth factors such as transforming growth factor (TGF)- β , epidermal growth factor and fibroblast growth factor (FGF)-2. Renal interstitial cells play an

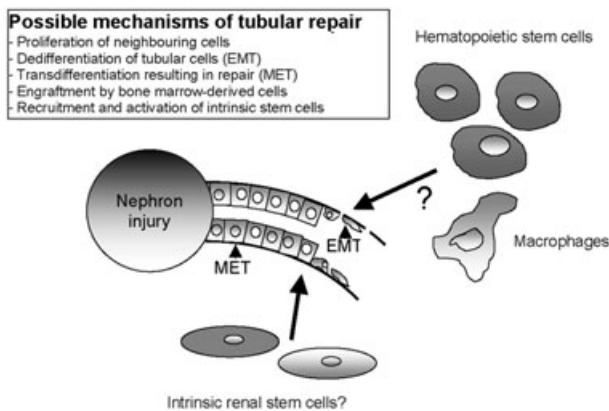


Fig. 1 Diagram of the putative events leading to tubular repair. Tubular epithelial cells may undergo epithelial–mesenchymal transition (EMT), where cells change their phenotype to a more immature/embryonic one in response to injury. Epithelial–mesenchymal transition is thought to be an essential component in the process of recovery following acute renal damage. In addition, bone marrow progenitor cells may home and integrate into repairing renal cells by a process of either transdifferentiation and/or fusion. A resident population of renal stem cells has not yet been identified in the adult kidney. Potentially these cells could also differentiate into renal-specific cell types upon activation. MET, mesenchymal–epithelial transition.

important role in both kidney morphogenesis and kidney regeneration following injury. Although the origin and precise function of adult interstitial fibroblasts remains unclear, it has been suggested that the recruitment of fibroblasts to the renal interstitium of the adult kidney during injury may support the repair of damaged tubules in an attempt to restore an embryonic environment.^{33,41–43} Fibroblasts are derived from the metanephric mesenchyme of the developing kidney, as are other epithelial components of the nephron. Conversely, there is increasing evidence that bone marrow-derived cells are progenitors for tissue fibroblasts.^{33,39,44,45} Using bone marrow chimeras and transgenic reporter mice, Iwano *et al.* provide evidence that interstitial cells are derived from both bone marrow and also tubular epithelial cells undergoing EMT during fibrogenesis.³³ Local proliferation of myofibroblasts during inflammation may then further contribute to ECM accumulation associated with the development of fibrosis. In a model of ureteral obstruction, approximately 36% of fibroblast-specific protein-1 (FSP-1)-positive fibroblasts coexpressed a LacZ marker for proximal tubular cells. A smaller proportion of FSP + ve, CD34-ve fibroblasts were found to migrate to the renal interstitium from bone marrow. These studies highlight the potential plasticity of differentiated cells in the kidney in response to activation and injury.

It is not known if the adult kidney contains a progenitor stem cell population capable of differentiating into different renal cell types. However, there is current belief

that most organs contain stem cells, but the tools needed to recognize them have not yet been developed.^{41,42} Stem cells are thought to reside in a ‘niche’, a local microenvironment that they occupy in an undifferentiated state, protected from environmental damage.⁴³ For example, it is thought that circulating bone marrow cells may home into tissue-specific niches that induce them to adopt the lineage fate of their new microenvironment.

Fusion

There is suggestion that the spontaneous fusion of cells may account for the apparent ability of bone marrow stem cells to produce non-haematopoietic lineages including kidney cells. It remains uncertain whether bone marrow cell engraftment represents a transdifferentiation or cell fusion process and if these events occur in an *in vivo* setting. Even so, caution must be taken when defining the biological relevance of stem cell plasticity in relation to possible cell fusion events.⁴⁶ These questions have been raised as a result of studies showing that bone marrow-derived cells can fuse with hepatocytes, cardiomyocytes and Purkinje cells, giving rise to products with a mixed genotype and phenotype.^{47–50}

Most studies demonstrating bone marrow cell plasticity use transplantation of marked bone marrow stem cells that are found to incorporate into the host tissue. These approaches do not distinguish cell transdifferentiation from cell fusion. However, other experimental approaches involving genetic and cytogenetic markers have demonstrated the ability of bone marrow cells to fuse with cells in the liver,^{48,51} brain,⁴⁹ heart,⁵⁰ and skeletal muscle.⁵² The precise mechanisms and identities of the cell types involved in bone marrow cell fusion are unknown, but studies using CD45-Cre or lysozyme M-Cre mice suggest that cells of the myeloid lineage may contribute to tissue repair by a cell fusion process.^{50,52}

There has been no evidence to date suggesting that renal cells undergo fusion with circulating extra-renal cells, but optimal methods for testing this phenomenon are still emerging. Masuya *et al.* used Y chromosome analysis in male-to-male mouse bone marrow transplantations to evaluate the possibility that cell fusions were key events in the observed plasticity of bone marrow-derived cells in the kidney.²⁶ Single GFP-labelled haematopoietic stem cells were found to reconstitute glomerular mesangial cells, but only a single Y chromosome was observed in each GFP-positive mesangial cells.²⁶ These studies suggest that the differentiation of bone marrow-derived cells into glomerular cells does not involve cell fusion. The biological significance of cell transdifferentiation versus cell fusion in repairing kidneys needs to be further investigated.

One concern for therapies that facilitate bone marrow cell fusion is that the resultant cell ploidy between transplanted and host tissue may have a high likelihood for

neoplastic transformation. This concern may be unjustified because cell fusion is thought to be a naturally occurring phenomenon in development as well as in tissue repair and regeneration.^{18,47,53,54} Further extensive investigation of the mechanisms of cell fusion as a means to produce healthy functional cells may also provide therapeutic potential relative to other new cell- and gene-based therapies.

REPARATIVE RENAL MEDICINE

Renal regenerative medicine includes the development of new stem cell-based therapies and tissue engineering that may offer alternatives for restoring or maintaining kidney function.⁵⁵ The progression of renal disease may not only involve renal cell loss but also defective renal repair mechanisms.³⁴ To date, attempts to accelerate renal recovery and functional repair have focused on the administration of growth factors such as epidermal growth factor, hepatocyte growth factor, and insulin-like growth factor.⁴⁰ Although these growth factors have been found to be important in repair following kidney injury, there has been no progress in the use of growth factors for accelerated recovery of patients with acute tubular necrosis.

Evidence demonstrating the plasticity of bone marrow stem cells provides an exciting opportunity to explore the use of allogenic or autologous cells in a regenerative approach for patients with renal disease. However, current reports of bone marrow cell engraftment rates in the repairing kidney have been observed in some instances to be <1%. An emerging field in stem cell biology is the identification of factors that control the differentiation, homing and integration of circulating bone marrow cells to injured kidney tissue.^{27,34,56}

It is an exciting possibility that adult-derived bone marrow cells or other potential progenitor cell populations could be used therapeutically in patients to aid in renal repair. Overcoming immune rejection in the development of new cellular-based therapies will present challenges, unless the cells are derived from the patients themselves. The long-term benefits of embryonic or adult stem cell engraftment in patients with renal disease are also unclear. It is likely that the success of stem cell therapy will be reliant on the timing of treatment in combination with established treatment strategies for patients with renal disease. Facilitation of endogenous renal repair processes and integration of stem cells into repairing kidneys will have significant therapeutic benefit only by controlling the initial inciting stimulus as well as the pro-inflammatory events leading to the progression of fibrosis. Stem cell therapy could also be used in combination with gene therapy to provide a novel avenue for the possible delivery of genes or growth factors to specific sites of injury in the kidney to aid in renal recovery. Immunosuppressive therapy would also have to be

considered in this setting because exposure of new proteins to patients may lead to immunological destruction of the new phenotype.⁵⁷

An alternative strategy to the transplantation of adult stem cells in renal regenerative medicine is the use of differentiated ES cells. The promise of ES cell therapy over autologous transplants of adult stem cells is the capability for producing an unlimited supply of tissue-specific terminally differentiated cells. The pluripotency and immortality of ES cells enable them to differentiate into cells of all three embryonic germ layers.⁵⁸ However, methods have not yet been elucidated that control the differentiation of ES cells towards mesodermal precursors of the adult kidney. Commonly raised questions for ES cell transplantation include concerns over immune rejection and the potential for the development of neoplasia.⁵⁹ The application of ES cells as a future renal replacement therapy for patients with renal disease will require a better understanding of immune rejection strategies and the control of ES cell differentiation into renal precursors.

Not to be overlooked in the development of future stem cell therapies is the existence of resident kidney cells that may potentially be able to repair injured tissue. A rapidly expanding field of renal stem cell biology is the study of nephrogenesis. The metanephric mesenchyme of the embryonic kidney can generate all epithelial cells of the kidney excluding collecting ducts. The study of kidney development may offer insights to identify novel markers for renal stem or progenitor cells. The possibility remains that immature stem cells left over from nephrogenesis remain in the kidney for postnatal renal regeneration and remodelling. Future identification and characterization of progenitor cells in the kidney and locations where they reside may prove imperative for the development of new therapeutic strategies. Studies of renal repair in cartilaginous and bony fish have demonstrated that they have the ability to produce new nephrons in response to loss.^{60,61} This production of new nephrons appears to recapitulate embryonic kidney growth and does not occur in adult mammals. A more complete understanding of how stem cells are involved in the production of nephrons in adult fish may open the way for triggering a similar response in adult human kidneys.

Finally, the field of tissue engineering involves the possibility of restoring renal function through isolation of cultured renal cells for expansion and organ reconstitution. Donor tissue can be dissociated to individual cells that are either transplanted directly, or expanded *in vitro*, for implantation on a support matrix.⁶² The implanted tissue can be derived from primary autologous cells, heterologous, or allogenic cells. Due to its complex structure and function, the kidney is regarded as a challenging organ for tissue engineering reconstruction.⁶³ Past studies using single suspensions of isolated and cultured mouse epithelial cells seeded onto biodegradable polymers, have successfully reconstituted nephron segments and reim-

planted them into syngeneic hosts.⁶⁴ In a more recent study, Lanza *et al.* used renal cells obtained through nuclear transfer and seeded them onto devices that were implanted into steer.⁶⁵ The seeded implants were found to form well-defined nephron structures that secreted dilute urine. These studies were the first to demonstrate the use of therapeutic cloning for replacement of transplantable kidney tissue *in vivo*.^{50,65}

CONCLUSIONS

There has been considerable excitement over the ability of bone marrow cells to cross lineage boundaries and engraft into a number of different tissue types including kidney. This evidence has challenged traditional views as to the source of cells contributing to cellular repair following acute renal damage. The process of transdifferentiation and/or cell fusion that may account for incorporation of bone marrow-derived cells into repairing kidneys needs to be further investigated. Further insight into the pluripotency of bone marrow cells and the potential of intrinsic renal cells may open the way for the development of new interventions based on acceleration of renal regeneration leading to restoration of renal function. The field of renal regenerative medicine that includes the development of new stem cell-based therapies and tissue engineering strategies may offer alternatives for renal replacement. In order for new cell- and/or gene-based therapies to become a therapeutic option, the source of stem cells, immune rejection tactics, and the 'window of opportunity' for patients with chronic renal disease will need to be further considered.

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