Hindawi Publishing Corporation
Journal of Biomedicine and Biotechnology
Volume 2012, Article ID 453519, 10 pages
doi:10.1155/2012/453519

Review Article

De Novo Kidney Regeneration with Stem Cells

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Received 13 June 2012; Accepted 16 October 2012

Academic Editor: Herman S. Cheung

Recent studies have reported on techniques to mobilize and activate endogenous stem-cells in injured kidneys or to introduce exogenous stem cells for tissue repair. Despite many recent advantages in renal regenerative therapy, chronic kidney disease (CKD) remains a major cause of morbidity and mortality and the number of CKD patients has been increasing. When the sophisticated structure of the kidneys is totally disrupted by end stage renal disease (ESRD), traditional stem cell-based therapy is unable to completely regenerate the damaged tissue. This suggests that whole organ regeneration may be a promising therapeutic approach to alleviate patients with uncured CKD. We summarize here the potential of stem-cell-based therapy for injured tissue repair and *de novo* whole kidney regeneration. In addition, we describe the hurdles that must be overcome and possible applications of this approach in kidney regeneration.

1. Introduction

The kidney is a complex tissue consisting of several different cell types including glomerular podocytes, endothelial cells, mesangial cells, interstitial cells, tubular epithelial cells, and connecting duct cells. These cell types interact to establish a precise cellular environment that functions as an efficient tissue. The *de novo* reconstruction of the kidney is a more difficult challenge than the regeneration of many other tissues because of its complicated anatomical structure. In recent years, regenerative medicine has made remarkable progress with various groups reporting that pluripotent stem/progenitor cells have the capacity to regenerate damaged renal tissue and improve kidney function in an experimental model. However, cell-based therapy such as stem cell injection for tissue repair is not effective for the terminal stage of chronic kidney disease (CKD), which is referred to as end stage renal disease (ESRD) because of the damage that has occurred to the complex structure of the kidney including its scaffold. Currently, CKD is a serious disease worldwide that causes high mortality because of increased cardiovascular risk. The terminal ESRD stage requires renal replacement therapy and the number of ESRD patients continues to increase because of the shortage of donor organs. Consequently, more than 290,000 ESRD patients are currently undergoing dialysis in Japan.

To address this growing clinical problem, we have made a partial kidney reconstruction from mesenchymal stem cells (MSCs) in an attempt to regenerate a whole functional human kidney. In addition we have investigated the regeneration of whole kidneys in animals. Nearly all of these studies have used pluripotent stem cells, and an artificial material, blastocysts or metanephroi to act as a scaffold for the stem cells. Here, we discuss the utility of stem cells including embryonic stem (ES) cells, induced pluripotent stem (iPS) cells, MSCs, and renal stem/progenitor cells, for the treatment of damaged renal tissue. In addition, we discuss the current advantages of *de novo* whole kidney regeneration and the obstacles that must be overcome before its clinical use is possible.

2. Embryonic Stem Cells

The first ES cells were initially derived from the inner cell mass of blastocyst-stage mouse embryos in 1983 [1]. These ES cells are pluripotent, have the ability to self-renew, and

can differentiate into several cell types of the mesodermal, endodermal, and ectodermal lineages [1]. Therefore, they have the capacity to be used as an effective tool for kidney regenerative therapy. The first human ES cell line was established by Thomson and colleagues in 1998 [2] and subsequently human ES cell lines have been found to be capable of differentiating *in vitro* into extraembryonic and somatic cell lineages [3]. If human ES cells are cultured with a mixture of eight growth factors (basic fibroblast growth factor (bFGF), transforming growth factor β1 (TGF-β1), activin-A, bone morphogenetic protein-4 (BMP-4), hepatocyte growth factor (HGF), epidermal growth factor (EGF), β-nerve growth factor (β-NGF), and retinoic acid) they will differentiate into cells expressing WT-1 and renin [4]. In addition, it has been shown that mouse ES cells stably transfected with Wnt4 will differentiate into tubular-like structures that express aquaporin-2 when cultured in the presence of HGF and activin-A [5]. The combination of LY294002, CCG1423, and Janus-associated tyrosine kinase inhibitor 1, was shown to enhance the differentiation of mouse ES cells into a pool of renal progenitor cells and intermediate mesoderm [6]. Steenhard et al. investigated an *ex vivo* culture system, in which ES cells were microinjected into the developing metanephros and this was cultured to determine the capacity of ES cells to differentiate into renal cells. They identified renal epithelial structures that resembled tubules with an efficiency approaching 50% and on rare occasions, individual ES cells were observed in structures resembling glomerular tufts [7]. In addition, when ES cells, treated with retinoic acid, activin A, and BMP-7, were injected into a developing metanephros, they contributed to the tubular epithelia with almost 100% efficiency [8]. The injection of ES cells with brachyury (T) expression into developing metanephros explants in organ culture, resulted in their incorporation into the blastemal cells of the nephrogenic zone. After a single injection into a developing, live, newborn mouse kidney, these cells were integrated into the proximal tubules with normal morphology and polarization of alkaline phosphatase and aquaporin-1 [9]. On the other hand, we recently reported that the *in vitro* culture of monkey ES and human iPS cells in rat metanephros showed teratoma formation [10]. In considering the therapeutic approaches using human ES cells two major issues arise. One issue is the ethical concerns surrounding the use of donated eggs to establish ES cells, and the other is the immune rejection due to histocompatibility antigenic differences between the ES cells and patients [11]. In summary, ES cells are a valuable cellular source for investigating the mechanism of cell development, but are unsuitable for clinical applied regeneration therapy.

3. Induced Pluripotent Stem Cells

Takahashi and Yamanaka have reported the generation of induced pluripotent stem (iPS) cells from murine somatic cells by retroviral transfer of expression constructs for the transcription factors Oct3/4, Sox2, Myc, and Klf4 [12]. Similarly, iPS cells have been established from several mammalian species, including rat [13, 14], rabbit [15], pig [16, 17], monkey [18], and human [19]. The generation of iPS cells has recently been reported from human mesangial cells [20], urine [21], and tubular cells [22]. In fact, iPS cells can be established without transfection of Klf4 [22] and Myc, which is a oncogenic factor [22, 23]. These data suggest that the oncogenic risk associated with iPS cell generation can be decreased by expressing only Oct3/4 and Sox2. Therefore, it is possible to prepare patient-specific pluripotent cells without manipulating germ cells because iPS cells are pluripotent and can be generated from adult somatic cells. Consequently, there are no ethical issues with the usage of iPS cells and immune rejection should not be a problem compared to ES cells. Potentially, iPS cells could provide a source of cells for kidney tissue repair or organ regeneration, although the difference between ES cells and iPS cells in their regenerative capacity to become kidney tissue has not yet been elucidated. The therapeutic potential of autologous iPS cells in a mouse model of hereditary disease has already been reported [24]. Therefore, the generation of iPS cells may open the door for a new autologous stem cell therapy for kidney regeneration. One recent study has indicated that the transplantation of iPS cells, but not ES cells, induces a T-cell-dependent immune response even in a syngeneic mouse [25]. These data contradict the concept of using iPS cells for regenerative medicine and therefore we need to evaluate the indications for iPS cells before they can be used in a clinical application.

4. Mesenchymal Stem Cells (MSCs)

Since 2000, bone marrow-derived stem cells (BMDCs) have been used in experimental kidney disease models because of their ability to differentiate into organ-specific cell types and regenerate several parts of the kidney. Several studies have indicated that treatment with BMDCs can ameliorate several injured renal tissues: tubular epithelial cells [26, 27], mesangial cells [28–30], podocytes [31, 32], and endothelial cells [33–35]. BMDC treatment can contribute to the attenuation of renal fibrosis during chronic renal disease progression [36]. However, donor BMDC migration into the kidney is very rare and their ability to transdifferentiate is limited. It is possible that the benefit of administering BMDCs is only derived from the paracrine action of the injected cells [37, 38]. Bone marrow includes hematopoietic stem cells (HSCs), MSCs, and endothelial progenitor cells and the use of selected populations of BMDCs such as MSCs has been proposed [39, 40]. The injection of bone marrow-derived MSCs can result in repair of the kidney and improve function in acute renal failure. Furthermore, several studies have shown that MSCs derived from kidney [41] and adipose tissue [42] instead of bone marrow represent a source of cells for the improvement of damaged renal tissue and function. These studies suggest that the presence of BMDC-derived kidney component cells is most likely due to the MSC population, which are adult stem cells with the capacity for self-renewal and multipotent differentiation. MSCs also produce cytokines such as vascular endothelial growth factor (VEGF), HGF, and insulin-like growth factor-1 (IGF-1) [43] that inhibit the profibrotic activity of TGF-β, which is a major factor for that epithelial-mesenchymal

transition (EMT) that leads to kidney fibrosis [44] and causes CKD. These humoral factors act to reduce inflammation and repair damaged kidney tissue. VEGF resolves glomerular inflammation, enhances glomerular capillary repair [45], induces endothelial cell proliferation, and prevents the loss of peritubular capillaries [46]. HGF inhibits epithelial cell death and accelerates regeneration and remodeling of damaged renal tissue [47]. IGF-1 secreted by MSCs accelerates tubular cell proliferation and aids the function and repair of injured renal tissue [48]. The conditioned media obtained from cultures of MSCs induces the migration and proliferation of kidney-derived epithelial cells and diminishes proximal tubule cell death [49]. These investigations show that the improvement of kidney function associated with MSC treatment is most likely caused by the secretion by MSCs of humoral factors that act on the injured tissue.

More recently, MSCs have been used in acute renal injury models but also the treatment of CKD [50, 51], diabetic nephropathy (DN) [52–55], and in a chronic allograft nephropathy model [56]. MSC treatment has also reduced renal fibrosis and ameliorated renal function in a rat remnant kidney model [50]. The levels of all cytokines in serum were decreased in MSCs-treated CKD rats, which suggests that MSCs therapy can indeed modulate the inflammatory response and suppress kidney remodeling in chronic kidney disease. In the same way, injected MSCs regulated the immune response that resulted in the acceleration of glomerular tissue repair and an improvement in kidney function in DN model rats [52–55]. It has also been observed that the injection of MSCs 11 weeks after kidney transplantation prevents interstitial fibrosis [56]. These data suggest that MSCs transplantation can inhibit the progression of DN and CKD and improve allograft renal function in both animals and humans. In contrast, MSCs can maldifferentiate into glomerular adipocytes accompanied by glomerular sclerosis [57] thus calling into question the benefit of long-term MSC treatment for chronic glomerular disorders [58]. Furthermore, recent reports suggest that the administration of external stem cells has additional risks in a clinical setting [59, 60].

Noh et al. reported that uremia induces functional incompetence of bone marrow-derived MSCs in an animal model [61]. Uremic MSCs showed decreased expression of VEGF, VEGF receptor 1, and stromal cell-derived factor (SDF)-1α, increased cellular senescence, decreased proliferation, defects in migration in response to VEGF, and SDF-1α and tube formation *in vitro* [61]. This study suggests that MSCs from CKD patients may be inappropriate as a source of cells for regeneration therapy. Further research is required to evaluate and solve the problems associated with regeneration therapy in order to make safe and effective use of MSC for kidney regeneration.

5. Renal Stem/Progenitor Cells

Adult stem/progenitor cells have been isolated from many adult organs that have clonogenic, self-renewing ability and will give rise to terminally differentiated cells of original tissue. Renal stem/progenitor cells exist in the adult kidney and are located in specific locations such as the renal papilla [62], tubular epithelial cells [63], Bowman's capsule [64], and the S3 segment of the proximal tubules [65, 66].

A number of different approaches have been made in the investigation of the functional role of renal stem/progenitor cells in the adult kidney [67]. The evidence for the presence of renal stem cells in adult kidney has relied upon the presence of cells positive for bromodeoxyuridine (BrdU), specific cell surface markers such as CD133 and CD24, or side population (SP) phenotypes. The first approach utilized a short pulse administration of BrdU followed by a long chase period. The rationale for this was based on the characteristically slow cycling time of organ-specific adult stem cells [68–70]. Stem cells incorporate BrdU into their DNA and retain this label to enable detection for an extended period of time [71, 72]. In normal rat kidney, cells that retain the BrdU label can be detected in papilla [62], as well as proximal, distal, and collecting tubules [63]. These cells proliferate in response to renal damage and differentiate into fibroblasts [73], proximal tubule and collecting duct cells as well as tubular structures *in vitro* [74]. Although more likely to represent stem cells, clonogenicity of these cells was not established [75].

An additional approach to studying renal stem/ progenitor cells is based on the analysis of stem-cell specific surface markers. Recent studies have been reported that a population of $CD133^+/CD24^+$ cells, in the absence of the podocyte marker, podocalyxin (PDX) are located at the urinary pole of the Bowman's capsule. This is the only place in the human kidney that appears to be contiguous with both tubular cells and glomerular podocytes [58, 64, 76–78]. Clonally-expanded $CD133^+/CD24^+/PDX^-$ progenitor cells are multipotent and are capable of differentiating into podocytes and tubular cells *in vitro* [76]. This population also contributes to the regeneration of podocytes and tubular cells after injection into mice with acute renal failure [58, 76].

Analysis of side population (SP) phenotypes has been adopted as another approach to identify renal stem cells in fractionated whole kidney. The term SP is used to describe HSCs that are isolated by using dyes such as Hoechst 33342 and Rhodamine 123 because HSCs have the ability to efflux these dyes. Cells with the same efflux profile in kidney may also a similar organ-based SP phenotype and function as organ-specific stem cells [75]. SP cells have been reported to present in the adult rodent kidney [79–82], and adult kidney SP cells show multilineage differentiation *in vitro.* The injection of adult kidney SP cells reduces renal damage without significant tubular integration [82, 83]. These data reveal that humoral factors may be important for amelioration of renal injury. However, it remains unclear whether kidney-derived SP cells are in fact renal stem cells, because their capacity for self-renewal has not been established [75].

Lindgren et al. recently demonstrated that aldehyde dehydrogenase (ALDH) activity can be used as a marker for isolation of cells with progenitor characteristics from adult human renal tissue [66]. Primary renal cortex cells with high ALDH activity were isolated by fluorescence-activated cell sorting (FACS) and express CD24 and CD133, which

are previously described markers of renal progenitor cells of Bowman's capsule. Functional and bioinformatic analyses of these cells showed that they have a robust phenotype that allows an increased resistance to acute kidney injury and suggests that these cells may spearhead the repopulation of renal tubules after injury.

A number of questions regarding the use of renal stem/progenitor cells in regenerative therapy remain to be answered. These include whether endogenous renal stem cells can be identified efficiently, whether they can be expanded *in vitro* and redelivered to a damaged kidney. Renal stem cell represents only 0.1% of the cells in an adult kidney [75, 82]. Therefore, whole kidney fractionation was necessary to produce sufficient renal stem/progenitor cells in these recent reports. Renal stem/progenitor cells differentiated from extrarenal stem cells such as MSCs, ES, and iPS cells may be promising cellular sources for kidney repair. However, a reliable method of inducing extrarenal stem cells to differentiate into renal progenitor cells has not been established at this time.

6. Other Stem Cells

Recent studies have reported that multilineage-differentiating stress-enduring (Muse) cells were isolated from human dermal fibroblasts. Muse cells are characterized by stress tolerance, expression of pluripotency markers, self-renewal. In addition, they have the ability to differentiate from a single cell both *in vitro* and *in vivo* into endodermal, mesodermal, and ectodermal cells [84]. Muse cells may also have the possibility to regenerate injured renal structure and further study of their use in regeneration therapy is required.

7. *De Novo* Organ Regeneration

7.1. Organ Regeneration Using Bioengineered Scaffolding. Advances in biomaterial engineering have produced bioengineered scaffolds that facilitate improved differentiation of transplanted cells. Tissue-engineering strategies combining artificial scaffolds and stem cells have been adapted for kidney regeneration. Lanza et al. initially reported that a histocompatible functional kidney was generated by using a specialized polymer tube as the artificial scaffold [85]. They used a nuclear transplantation technique in which dermal fibroblasts isolated from an adult cow were transferred into enucleated bovine oocytes and then transferred nonsurgically into progestin-synchronized recipients. Metanephroi from embryos were digested using collagenase, and the cells were expanded *in vitro* until the desired number was produced. The cells were then seeded onto a specialized polymer tube, which was implanted into the same cow from which the cells had been cloned. This renal device that was seeded with cloned metanephric cells appeared to produce a urine-like liquid. Histologic analysis showed that the device had well-differentiated kidney-like construction. This included organized glomerulus-like, tubular-like, and vascular elements, which were clearly distinct from each other, but were continuous within the structure. The kidney-like structure appeared to be integrally connected in a unidirectional manner to the reservoirs, resulting in the excretion of urine into the collection system. This study established that bioengineered tissue scaffolds are potential tools for kidney regeneration.

7.2. Organ Regeneration Using Decellularized Cadaveric Scaffolds. Recent studies have reported that a decellularized organ can be useful as an artificial scaffold. The decellularization process preserves the structural and functional characteristics of the native microvascular network. Ott et al. showed the successful development of a functional artificial rat heart using a decellularized cadaveric heart as the artificial scaffold [86]. A whole-heart scaffold with intact three-dimensional geometry and vasculature was prepared by coronary perfusion with detergents into the cadaveric heart. This heart was then colonized by neonatal cardiac cells or rat aortic endothelial cells and cultured under physiological conditions to promote organ development [86]. The injected neonatal cardiac cells produced a contractile myocardium, which performed the stroke function.

Cadaveric scaffolds have also been investigated to develop transplantable livers and lungs using mature hepatocytes and alveolar epithelial cells, respectively [87, 88]. After transplantation of the recellularized grafts, they successfully functioned as hepatocytes and gas exchangers, respectively. This type of approach is promising for regenerating organs that have a simple architecture.

Based on a series of studies, Ross et al. successfully regenerated an entire kidney using a decellularized cadaveric kidney scaffold [89]. After decellularization of an intact rat kidney, murine ES cells were injected into the renal artery where they localized in the vasculature, glomeruli, and tubules. Immunohistochemical analysis indicated that the injected ES cells had lost their embryonic appearance and had developed to mature kidney cells. This approach was supported using the primate kidney [90] but the regenerated primate kidney did not have sufficient renal function to produce urine and erythropoietin (Epo). Therefore the reconstruction of a whole functional kidney may be difficult using this approach.

8. *De Novo* Organ Regeneration Using Blastocyst Complementation

Recently, a dramatic advance has been made in pancreas regeneration using the interspecific blastocyst injection of iPS cells [91]. When rat iPS cells were injected into $Pdx1^{-/-}$ (pancreatogenesis-disabled) mouse blastocysts, the newborn rat/mouse chimera possessed a pancreas derived almost entirely from rat iPS cells. This result shows that when an empty developmental niche for an organ is provided, then iPS cell-derived cellular progeny can repopulate that niche and can develop into the missing contents of the niche. In fact, they can form a complicated organ that is composed almost entirely of cells differentiated from donor iPS cells, even if the blastocyst complementation is derived from a different species.

Espejel et al. generated chimeric mice in which all of the hepatocytes were derived from iPS cells from blastocysts with

fumarylacetoacetate hydrolase deficiency [92]. The entire liver was composed of iPS cell-derived hepatocytes by the time the mice reached adulthood. iPS cells have the intrinsic ability to differentiate into fully mature hepatocytes that provide full liver function. The iPS cell-derived hepatocytes also replicated the unique proliferative capabilities of normal hepatocytes.

This blastocyst complementation system was recently applied to whole kidney reconstruction [93]. Murine iPS cells were injected into blastocysts from mice that did not express the SAL-like 1 (Sall1) zinc-finger nuclear factor essential for kidney development. The newborn mice possessed kidneys derived almost entirely of injected iPS cells. While this is an attractive system, it is not available for clinical use because it is impossible to generate the vascular and nerve systems. In addition, immunohistochemical analysis of the regenerated kidney indicated that the renal vascular system including renal segmental, lobar, interlobar, arcuate, and interlobular arterioles was a chimeric structure originated from both host cells and donor iPS cells [94]. When rat iPS cells were injected into Sall1-null mice blastocysts, they did not generate rat kidneys in mice. This suggests that the key molecules in mice involved in the interactions of the mesenchyme and the ureteric buds do not cross-react with those in rats. Therefore, to generate xenoorgan using xenoblastocysts, it would be necessary to generate a host animal strain lacking all of the lineages that contribute to the kidney [93]. At present the most important ethical issues involved with manipulating heterogeneous blastocysts containing iPS cells remain unresolved. In addition, while it is quite difficult to generate interspecific chimeras in animals, blastocyst complementation appears to be one of the most promising strategies for regenerating the kidney.

9. *De Novo* Organ Regeneration Using the Metanephros of Growing Xenoembryos

The embryonic metanephros is a primordium of the adult mammalian kidney and represents a source for a transplantable artificial kidney [95–99]. Metanephroi implanted into a host renal cortex or omentum continue to develop and enlarge. The differentiated metanephroi in a host animal have vascularized glomeruli and mature proximal tubules and produce urine [95, 96]. After an intact ureteroureterostomy, anephric rats with a transplanted metanephros show prolonged lifespan [96]. The transplanted metanephros is also metabolically functional and produces Epo and renin, as well as elevates the blood pressure of the host animal [100, 101]. Furthermore, porcine metanephroi transplanted into the omentum of mice treated with costimulatory blockade [97] or transplanted under the kidney capsules of immunodeficient mice [98], also differentiated into a functional nephron. The levels of urea nitrogen and creatinine were higher in the cyst fluid produced by the transplanted tissue, than in the sera of the transplanted mice [98]. This suggests that the metanephros is a potential source of transplantable regenerated kidney to address the shortage of organs for kidney transplantation.

We have attempted to regenerate a whole functional kidney using a developing heterozoic embryo as an organ factory. We sought to use this mechanism of a developing embryo by applying the stem cells at the niche of organogenesis. During development of the metanephros, the metanephric mesenchyme (MM) initially forms from the caudal portion of the nephrogenic cord [102] and secretes glial cell line-derived neurotrophic factor (GDNF), which induces the nearby Wolffian duct to produce a ureteric bud [103]. Therefore, we microinjected GDNF-expressing human MSCs (hMSCs) into the site of budding. The recipient embryo was grown in a whole embryo culture system, and the metanephros that formed was developed in organ culture [104, 105]. Virus-free manipulation can also be performed using thermoreversible GDNF polymer [106]. Donor hMSCs were found to be integrated into the rudimentary metanephros and morphologically differentiated to tubular epithelial cells, interstitial cells, and glomerular epithelial cells [104]. These data indicate that using a xenobiotic developmental process for growing embryos allows endogenous hMSCs to undergo an epithelial conversion and develop into an orchestrated nephron including glomerular epithelial cells and tubular epithelial cells. The hMSCs can also differentiate into renal stroma after renal development [104].

We then examined whether there was urine production from the "neokidney," which is of major importance for successful *de novo* renal regeneration. Urine production requires that the new kidney has the appropriate vascular system of the recipient. Therefore, we transplanted metanephroi into the omentum in order to allow for vascular integration from the recipient to form a functional nephron. As a result, an hMSC-derived neokidney was generated that contained a human nephron and the vasculature from the host [105, 107]. In addition, the neokidney produced urine that showed higher concentrations of urea nitrogen and creatinine than the sera of the recipient. This suggested that the neokidney that developed in the omentum was capable of producing urine by filtering the recipient's blood [107]. Furthermore, the hMSC-derived neokidney secreted human Epo, which was stimulated by the induction of anemia in the host animal, indicating that this system preserves the normal physiological regulation of Epo levels [108].

The current system we have developed may not reconstruct derivatives of the ureteric bud. Thus we sought to determine whether MSCs can differentiate into the ureteric bud progenitor using chick embryos. The hMSCs that expressed Pax2 were injected into the chicken ureteric bud progenitor region and they migrated caudally with the elongating Wolffian duct [109]. The hMSCs were integrated into the Wolffian duct epithelia and then expressed LIM1, revealing that they can differentiate into the Wolffian duct cells under the influence of local xenosignals [109]. These results indicate it might be possible to rebuild the whole kidney by transplanting hMSCs at a suitable time and place to regenerate derivatives of the MM and ureteric bud.

We recently reported that the xenotransplanted metanephros provides a niche for endogenous MSC differentiation into Epo-producing tissue [110]. Xenotransplanted metanephros, from rat into mouse and similarly from pig

into cat, expresses Epo of the host animal origin, as shown by PCR using species specific primers and sequence analysis. This suggests that there has been recruitment of host cells and Epo production. The Epo-producing cells were not differentiated from integrating vessels because they did not coexpress endothelial markers. Instead, Epo-producing cells were revealed to be derived from circulating host cells, as shown by enhanced green fluorescent protein (EGFP) expression in the grown transplants of chimeric mice bearing bone marrow from a transgenic mouse expressing EGFP under the control of the Epo promoter. These results suggest that donor cell migration and differentiation in a xeno-transplanted developing metanephros may be consistent between species. The Epo-producing cells were identified as MSCs by injecting human bone marrow-derived MSCs and endothelial progenitor cells into NOD/SCID mice. Furthermore, using metanephroi from transgenic ER-E2F1 suicide-inducible mice, the xenotissue component could be eliminated, leaving autologous Epo-producing tissue. Our findings may alleviate adverse effects due to long-lasting immunosuppression and help mitigate ethical concerns. These data suggest that xenometanephroi can provide the niche for host bone marrow cells to differentiate into Epo-producing tissues and they can be reconstructed to consist exclusively of host cell components using fate-controlled animals.

10. *In Vitro* Kidney Regeneration without any Scaffolding

A number of research groups are investigating whether pluripotent stem cells can differentiate into a kidney structure without any external scaffold. ES or iPS cells have been differentiated into mature cell types in adult organs, such as the pancreas [111, 112], liver [113, 114], and intestine [115] by using stepwise protocols mimicking the mechanism of embryonic development. In order to regenerate insulin-producing cells, ES [111] or iPS cells [112] were first differentiated into definitive endoderm, then foregut endoderm, followed by pancreatic progenitors, and eventually insulin-expressing endocrine cells. On the other hand, recent studies have revealed that autonomous formation of three-dimensional adenohypophysis [116] and optic cap [117] structures in aggregate culture of pluripotent ES cells. Osafune et al. previously established that a single cell from the MM, which highly expresses Sall1, can form colonies and reconstruct a three-dimensional kidney structure composed of glomeruli and renal tubules [118]. A recent study also established a novel method in which embryonic kidneys are dissociated into single-cell suspensions and then reaggregated to form organotypic renal constructions [119]. These investigations suggest the possibility of establishing a whole kidney from pluripotent stem cells by using the stepwise differentiation approach. This would involve initially directing the pluripotent stem cells to form intermediate mesoderm, then renal progenitors [11]. As a result the three-dimensional kidney structure could also be developed from these pluripotent stem cells *in vitro*.

The signals involved in embryonic kidney development have not yet been fully revealed and the technique required for the induction of iPS cell differentiation into renal cells remains uncertain at this time. Furthermore, the route for the reconstruction of the renal vascular system between the regenerated kidney and the recipient remains unclear. Therefore, this area requires additional research and further advances in stem cell biology will enable the development of new therapeutic strategies for the treatment of renal diseases.

11. Conclusions

We have summarized recent advances in renal regenerative therapy including the potential of stem cells to treat damaged renal tissue and to regenerate a whole organ *de novo*. At this time, the utilization of stem/progenitor cells for regeneration therapy has both advantages and disadvantages. Even though ES cells are pluripotent, there are ethical problems associated with the manipulation of germ cells in producing ES cells. Similarly, iPS cells are pluripotent but the use of retroviral transduction and our limited understanding of its effects hinder the clinical potential of iPS cells. The use of renal stem/progenitor cells in kidney regeneration is limited by their restricted growth and differentiation potential as well as their low prevalence. Therefore, renal stem/progenitor cells appear to be unsuitable for whole kidney regeneration. In contrast, MSCs are easily accessible, especially from adipose and do not require technical manipulations. However, MSCs from CKD patients may be inappropriate for regeneration therapy, because uremia induces functional incompetence of MSCs. On the other hand, recently, new findings against this opinion have been reported [120]. The determination of the optimal source of cells for *de novo* kidney regeneration remains an important aim.

On the other hand, we make effort to regenerate *de novo* a whole functional kidney by using xenoembryos and have investigated successful reconstruction of a part of a functional kidney derived from hMSCs, because of the necessity of *de novo* development of an entire functional organ for ESRD patients. Based on this success, we are currently investigating whether the pig is suitable for our system, because the porcine kidney is almost the same volume as the human kidney [98]. Even though kidney regeneration using heterologous animals, such as xeno-decellularized cadaveric organ, xenoblastcyst, and xeno-embryos, is a promising strategy, the ethical issues remain controversial. However, we hope that this system in larger animals will facilitate the development of larger organs that are more suitable for use in humans and make effort to solve the shortage of organ donors.

Acknowledgment

This work was supported by a Grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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