Cell therapy for renal regeneration—time for some joined-up thinking?

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How the kidney copes with the task of regenerating its parenchymal cells in baseline conditions, or after damage, is relatively poorly understood—particularly in comparison with the gastrointestinal tract, skin, hair follicle and cornea. There are, however, recent reports that the ‘rescue’ of tubular epithelium in models of hereditary tyrosinaemia 1 [1] and of podocytes in mice that model Alport syndrome [2,3] utilizes cells derived from experimentally grafted bone marrow. As this procedure is relatively simple, it may be that some will consider trying it in humans, but the mechanisms responsible are complex and poorly understood, and there are precious few reproducible results in rodents. It may be that cell therapies can eventually be developed, but our thinking about these approaches needs to be joined up, especially if the rescue relies upon cells joining together imperfectly.

Understanding the basics of stem cell biology may provide the key to new treatments for renal damage—as it has for the cornea and for the skin. For example, effective new treatments are possible for individuals with chemical burns to the cornea by isolating from the limbus of the contralateral eye a small number of stem or ‘master’ cells, from which the differentiated cells of the cornea derive, followed by propagation in vitro and then re-seeding [4]. There are strategies also for reducing the severity of forms of epidermolysis bullosa by targeting skin stem cells to achieve long-term expression of therapeutic gene constructs [5].

So what about renal stem cells? Most researchers agree that the kidney should likely possess stem cells responsible for the generation of many of the great variety of differentiated cells that are present in the adult kidney [6], but evidence for functional renal stem cells within adult mammals remains elusive, and their regenerative ability is incomplete, restricted mostly to replenishing some tubular epithelium. In contrast, fish such as the skate [7] and even the common goldfish [8] can generate entire new nephrons in response to damage.

The search for renal stem cells has led some to identify them tentatively as cells within the renal papilla that can retain a DNA label for several months, but these cells are likely to be not all stem cells, because many divided and lost their label when challenged by ischaemic injury [9]. Epithelial progenitors that exhibit some properties of stem cells have been cultured in vitro from tubules of rabbit [10] and those from rat contribute to tubular regeneration after ischaemia/reperfusion injury [11]; such cells from human kidney have been trialled in a type of renal-assist device [12]. A non-haematopoietic population of CD133+ cells has been isolated from human kidney, cloned in vitro and found able to contribute to tubular regeneration in severe combined immune deficiency (SCID) mice [13]. In addition, cells with attributes of mesenchymal stem cells (including differentiation into fat and bone) have been cultured from glomeruli and whole kidneys of mice [14] although their ability to generate epithelial cell types was not explored. A possible explanation for adult mammalian kidneys being unable to generate new nephrons is that the necessary stem cell population migrates away to the bone marrow (BM) along with haematopoiesis, whereas species that can make new nephrons host haematopoietic stem cells in their renal interstitium [15]. This hypothesis is supported in part by the presence in tubules of appropriately differentiated epithelial cells that are of extra-renal origin, e.g. the epithelial nucleus bears an unexpected Y-chromosome in either a male recipient of a female renal allograft, or in a female recipient of a male bone marrow graft [16]. Laser capture microdissection and analysis of a neutral genetic marker indicated that renal epithelium can be derived from extra-renal precursors in 88% of graft recipients [17]. So, perhaps, these studies allow us to detect a natural process that regenerates tubules (imperfectly) that is invisible unless a graft-derived marker is available.
There has been concern and debate (reviewed in [18]) over the validity of particular methods for detecting the presence of BM-derived epithelium, a particular concern where expression of cytoplasmic β-galactosidase has been used as a marker of cell origin in animal models. There is, however, an agreement over the origin of renal vascular endothelium; recent studies [19,20] have endorsed much earlier reports (reviewed in [21]) that the established endothelium within a graft persists, but damage can be repaired by endothelium derived from circulating endothelial precursors originating from the bone marrow.

What about other renal cell types? There is strong evidence for the extra-renal origins of mesangial cells based on the transfer by BM grafting of an appreciable disease phenotype to the glomerulus (including abnormalities in mesangial cells cultured from recipient mice) [22], and in rats a Thy1 Ab/nephrectomy-induced glomerulosclerosis was attenuated by whole BM grafts that generated mesangial and endothelial cells [23]. In addition, our group and others have reported that renal myofibroblasts [24,25] and podocytes [16,26,27] can bear markers of BM origin.

There are several important questions to ask about these non-endothelial BM-derived cells. First, are they derived from haematopoietic stem cells (HSCs) or mesenchymal stem cells (MSCs) or, in the case of fibroblasts, from circulating fibrocytes produced in the bone marrow? There are no absolute answers, as various groups of researchers hold particular cell types responsible in different models [28–30] and engraftment of kidney has not been detected after administering single cells [31].

Secondly, are they useful when they get there? In mouse models where BM is grafted weeks or months before induction of renal injury, the majority of tubular epithelium remains of indigenous (recipient) origin, almost irrespective of the type of injury and its extent, with a moderate percentage of cells bearing a marker of extra-renal origin. Nevertheless, these cells appear to have differentiated appropriately, bearing phenotypic markers just like their indigenous neighbours, so they appear useful as an extra cell in the right place at the right time while recovering from injury, even if only helping to maintain tubule integrity.

Thirdly, can their numbers be increased? A few studies have looked for evidence of the BM-derived cells responding as their neighbours to proliferative drives; in one, looking at the recovery phase after folic acid-induced acute tubular necrosis in mice, the BM-derived tubular cells were seen to enter S-phase (to divide), at least as often as their indigenous neighbours [32], but it is not known whether such cells survive long term. Strategies to mobilize BM in vivo vary considerably in their reported effects on engraftment and renal function [27,32–34], although several groups including ours are looking for any effects of co-administered growth factors and cytokines such as hepatocyte growth factor (HGF), epidermal growth factor (EGF) and erythropoietin (EPO).

Fourthly, how do they come to be formed? Various schemata have been proposed in which individual cells (whether stem cells or not) change fate, becoming re-programmed to accomplish the tasks appropriate to their new environment, but the mechanism to consider above all is that of cell fusion (see e.g. [35,36]). The reason being that cell fusion is established as the mechanism accounting for the dramatic rescue from liver failure of mice with a genetically defined deficiency in fumaryl acetoacetate hydrolase (Fah) [37]. Substantial engraftment of Fah-null liver by Fah-expressing hepatocytes is due to fusion of HSC-graft-derived myelomonocytic cells with resident hepatocytes, generating chimeric cells with a strong selective advantage that then multiply (reviewed in [38]).

Could renal disease or toxic injury be treated with a cell therapy? Potentially, yes, but there are immense gaps in comprehending how apparently successful studies have worked and in understanding what the consequences would be of generating cells that are not quite what they seem.

Some studies in mice report renal benefits of ‘cell therapy’ without being able to detect any of the administered cells in the affected tissue—a humoral or cytokine effect being considered responsible [27,39]. This may be perfectly correct, but it raises further concerns—where do the injected cells go, and what do they do there? It seems, even for quite different cell types, that ‘seeding’ can be widespread [31], and cells need not integrate within existing structures [40].

To try to discover if cell fusion contributes to the generation of BM-derived tubular cells, our group used confocal microscopy to count X- and Y-chromosomes in regenerating tubules of BM-grafted mice; we found no cells with the XXXY predicted from simple fusion, but did observe XXY cells, suggestive of fusion, and perhaps with other processes occurring [32,36,41].

Should we be concerned about cell fusion? It is, after all, a natural process, occurring frequently in biology as a way to increase the amount of cell membrane available to a cell committed to a restricted set of tasks. Around a third of the cells that are born in the nematode Caenorhabditis elegans undergo cell fusion; human placenta is covered with a syncytium formed by fusion of cells derived from subjacent progenitors, our muscle fibres are built from myotubes containing many nuclei and we rely on osteoclasts (arising by fusion of precursor cells of the monocyte/macrophage lineage) for bone degradation and aspects of the maintenance of the HSC niche [36,42,43]. So cell fusion is an essential part of life, and it has been used therapeutically in individual mice of a strain that suffers an abnormality of bone resorption (osteopetrosis, among other problems) [44]. Several years ago it was speculated that ‘Once the signals and underlying mechanisms are better understood, adult bone marrow-derived cells could be genetically engineered to deliver genes to specific targets, giving rise to a new form of gene therapy’ [45]. Since then, several reports have appeared indicating that the generally
low-frequency fusion events seen in graft recipients can follow pathways that alter cell phenotype and programming, but might not be completely benign processes.

We should consider the implications of the very elegant study reported by Held et al. [1], who were able to generate a renal phenotype in the Fah-null mouse mentioned earlier. Their strategy used a second defect introduced into the same tyrosine metabolic pathway to help generate substantial renal damage, but in a way that encouraged the survival of renal tubular cells derived from wild-type BM cells. Substantial replacement of Fah-null with Fah-expressing renal epithelium was seen, up to 50% when whole BM was grafted prior to selection. This level of engraftment might raise hope for strategies to correct genetic disease affecting tubules, but the authors found evidence that the genome of these ‘new’ cells could not be normal—de novo expression of the Fah-enzyme had occurred in tubular cells that possessed the host’s Y-chromosome. Thus, it seems some genetic exchanges had occurred between the nuclei of fused cells, followed by nuclear reduction. A similar mechanism is described elsewhere; e.g. the immensely complex Purkinje cells in the cerebellum of humans and mice (where entire nuclei appear to be exchanged) [46], and the eGFP+ hepatocytes generated in non-obese diabetic (NOD)-SCID mice from injected lentivirally-eGFP-tagged human cord blood HSC, where resulting green fluorescent hepatocytes (Figure 1) were unstable mouse:human heterokaryons, some with just a few human centromeres [47]. It may be that the gamma irradiation used in most studies to ablate recipient BM encourages an abnormal type of fusion resulting in altered gene copy number translocation and long-term sequelae; if so, perhaps conditioning regimes might offer a safer approach.

Very few laboratories have the resources to attempt studies of the complexity and duration of those on the Fah-null mice mentioned earlier, but perhaps strains of Alport mice will provide a more accessible and relevant model for testing cell therapies? A few studies have been reported, but already there are differences in the results found and mechanisms thought to be involved. First, Katayama et al. [48] indicated that wild type BM transplantation improves survival of Alport mice, but no definitive report of that abstract has yet appeared. In contrast, Sugimoto et al. [2] recently reported that grafting whole wild-type BM into young mice lacking the expression of the α3 chain of procollagen IV leads to an astonishing partial restoration of expression of the missing collagen chain (with incorporation of α3, α4, α5 triple helices in renal basement membranes), expression of α3 chain mRNA by podocytes, accompanied by improved architecture of the glomerular basement membrane (GBM) and, importantly, improved renal function [2]. Their results are especially remarkable in that renal injury was already established at the time of ‘rescue’ with unaffected BM. By comparison, results from our own group, also presented at the European Renal Cell Study Group, Dublin 30 March – 2 April 2006, using an Alport model, indicated that renal function and survival was better in recipients of normal BM than

Fig. 1. Reproduced with permission of the copyright holder AlphaMed Press [47]. Cell fusion, followed by nuclear fusion, with sorting and the selective loss of chromosomes are thought to have contributed to the generation of hybrid cells; synkaryons in which a mouse cell nucleus contains only a part of the human genome (red human centromeres). Hepatocyte-like eGFP+ cells containing few human centromeres. FISH for human centromeres highlights multiple spots and clusters (red) in human hepatocytes (A) but not in mouse hepatocytes (D). eGFP+ hepatocyte-like cells in experimental animals injected with human cord blood CD34 cells (B–F) contain only few human centromeres (C, F: high magnification). Nuclei were counter-stained with DAPI (blue).
Alport BM, but that there was a low level of neo-expression of the missing collagen gene as assessed by immunohistochemistry, RT-PCR, and in situ hybridization [3]. We were able to visualize candidate BM-derived podocytes (Figure 2), but given their scarcity, it seems unlikely that they alone account entirely for the improved renal function. It is tempting to consider that any benefits of BM in Alport mice are derived from the HSC compartment because we found MSC ineffective [3], and Ninichuk et al. [49], using an Alport mouse with more rapid disease progression, showed that MSCs, injected weekly, reduced interstitial fibrosis and loss of peritubular capillaries but did not incorporate into glomeruli or improve renal function or survival.

A major concern for all cell therapies, namely that establishing the neo-expression of a protein which had been missing is likely to induce an immune response, applies whether the rescue mechanism is via fusion or not. If the repaired podocytes in Alport mice are derived from fusion, there should still be a great deal of attention paid to the implications of this, before moving to clinical studies. What would be the consequence of podocytes made with unusual chromosomal composition? Would potential problems develop quickly, or only after months or years, and in which case would the benefits outweigh the risks?

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