Will fish foster regenerative medicine in man?

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Summary of the key findings

In the February 3, 2011 issue of Nature, an elegant paper by Diep et al. [1] provided important clues on the cellular source that contributes to the regeneration of new functional nephrons during zebrafish growth and after injury. They first demonstrated partial restoration of the nephron function after gentamycin-induced injury, suggesting that some nephrons recovered in the kidney of adult zebrafish. To identify the cellular source of nephron regeneration, they used a transplantation assay based on immunocompromised (by radiation) nephrotoxin-injected recipient fish that received unpurified donor whole-kidney marrow cells tagged with different fluorescent proteins. Donor whole-kidney marrow cells, mostly comprising non-tubular interstitial cells, gave rise to functional nephrons which developed even in areas distant from the injected site and successfully integrated into the recipient’s renal tubules. Serial transplantation of donor whole-kidney marrow cells revealed that nephron progenitors were long-lived and bore robust self-renewing capacity. Only cell aggregates but not single nephron progenitors formed new nephrons as indicated by transgenic lineage-tracing experiments. In vivo imaging of nephrogenesis, performed on optically transparent larval zebrafish, confirmed that cell aggregates arose from the coalescence of three or four cell types that expanded to form a renal vesicle, which subsequently elongated into a nephron (Figure 1). Microarray analysis was performed to determine how similar zebrafish progenitor cells were to mouse cap mesenchyme cells, which represent the multipotent, self-renewing progenitor population giving rise to podocytes, proximal and distal tubules during mammal kidney development [2]. A comparison of differentially expressed genes revealed that, despite having distinct molecular identities, the two cell populations shared a core set of regulatory genes conferring renal stem/progenitor cell potential.

Review of the field

The capacity for the kidney to generate new nephrons, throughout life, also called neonephrogenesis, is a common feature of fish which takes place in a peculiar site of the adult kidney, the neonephrogenic zone, containing aggregates of mesenchymal stem cells presumably deriving from a pool of embryonic stem cell-like cells [3]. In fish, neonephrogenesis is instrumental to sustain physiologic renal growth and to repair the kidney after damage as documented by the generation of new nephrons after reduction of renal mass [4]. Neonephrogenesis does not occur in mammals [5]. In rodents, kidney development continues on a few days after birth, whereas in humans, it stops at the end of gestation. Ablation of the kidney tissue does not elicit regenerative responses in mammals, possibly because, unlike fish, they do not possess the nephrogenic zone. Under normal physiological conditions, the mammal kidney has a low cell turnover, whereas after injury, increased cell proliferation drives tissue repair. Studies have been undertaken to establish which cell type promotes renal repair in mammals and whether reparative programmes depend on renal progenitor cells. Cells expressing the stem cell marker CD133, localized in the tubular interstitium of healthy human renal cortex, have been injected into mice with glycerol-induced acute kidney injury. From which, the cells differentiated, in vitro, into renal epithelium and endothelium, and enhanced the recovery from tubular damage in the mice [6]. A tentative progenitor cell population with a scattered distribution in the epithelial layer of the proximal tubules has been recently described in human renal cortex as expressing CD133, CD24, vimentin and cytokeratin 19 and the anti-apoptotic protein BCL2. Prominent regeneration of tubular epithelium after acute tubular necrosis showed the presence of CD133+/vimentin+ cells, confirming that these cells might represent progenitors [7].

Multipotent progenitor cells expressing CD133 and CD24, a molecule expressed by unduced metanephric mesenchyme during kidney development, have been isolated from Bowman's capsule of adult human glomeruli. Cells negative for podocyte markers are multipotent, generate podocytes and tubular cells in vitro and contribute to tubular cell regeneration after injection into mice with acute renal failure [8,9]. Lineage tracing of parietal epithelial cells in transgenic mice allowing for the demonstration that parietal epithelial cells migrate into the glomerular tuft and become podocytes, suggesting that these cells are responsible for podocyte renewal [10]. Available data can be interpreted to indicate...
that renal progenitors in adult mammals are there to drive renal regeneration initially replacing modest loss of functioning tissue. However, should the damage be severe enough, their reparative capacity is tipped in favour of dysregulated proliferation paving the way for generating hyperplastic lesions [11]. On this line, recent preliminary evidence describe the presence of stem/progenitor cells expressing the marker of metanephric mesenchyme NCAM and CD24 in rat Bowman’s capsule which chaotically proliferate and migrate in the glomerulus in disease conditions and are kept quiescent by angiotensin converting enzymes inhibitors [12]. Renal papilla has also been proposed as a niche for kidney stem cells because of the presence of large numbers of bromodeoxyuridine -retaining cells in the papillary interstitium in rats and mice [13]. Much remains to be explored as regarding the pathways which regulate stem/progenitor activation. Specifically, the method to unmask or reactivate renal progenitor cells and induce them to acquire a reparative phenotype still remains an open issue. It is likely that, to answer these questions, the zebrafish model could be of great help. An issue to be faced in fish is whether the multiple cell progenitors observed in the regenerated nephrons derive from a single cell clone or from multiple progenitors. The study of the pathways involved in the regulation of nephrogenic aggregates will allow for the possibility to set up strategies in order to stimulate and reactivate their counterpart, if any, in the mammalian adult kidney. It is currently unknown whether nephrogenic aggregates, similar to those described in the zebrafish, are present in the mammal kidney and if they could be activated. In this context, the simple vertebrate model of the zebrafish might represent a valuable tool to help understand whether kidney regeneration could be fostered in mammals and the mechanism behind this. It is conceivable that mixed populations of embryonic renal cells are needed for the formation of renal tissue in mammals as suggested by studies based on dissociation and reaggregation of embryonic kidney [14]. One key question that is not addressed by the paper of Diep et al. is the nature of cell aggregates that differentiate into nephrons. In vitro culturing and differentiation of purified clonally isolated cells will be a tool to understand the dynamics of regenerating systems germane to potential regenerative therapies. Finally, the recent observation of similarities between adult zebrafish heart and immature mammal heart which can regenerate after partial surgical resection, a capacity lost as age increases [15], raises the possibility that mechanisms underlying heart fish regeneration may be switched on in complex vertebrate not only in the heart but also in the kidney.

Take-home message

The zebrafish represents a simple vertebrate model which helps us to understand the mechanisms behind the activation of kidney regeneration in mammals. Unravelling the molecular identity of cell aggregates responsible for nephron regeneration and the pathways to regulate them will allow to reprogram or activate the bulk of renal progenitors that are present in the kidney of different mammal species. The future of renal regenerative medicine will mostly depend on fish. We have been already told that the philosophers would not have existed without them [16].

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References


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![Fig. 1. Schematic representation of in vivo nephrogenesis in zebrafish. Aggregates of three to four lhx1a+ mesenchymal cells (a) lying the pronephric tubule expand to form a renal vesicle which also expresses wt1b (b). The renal vesicle subsequently elongates into a nephron containing wt1b+ cells in the glomerulus and proximal tubule, pax8+ cells in the distal tubule and lhx1a+ cells located at the point of fusion with the pronephric tubule (c).](https://academic.oup.com/ndt/article-abstract/26/7/2107/1896946/fig-1)
Renal stress in vivo in real-time—visualised by the NGAL reporter mouse*

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Topic and summary of key findings

Acute kidney injury (AKI) is a serious syndrome in hospitalized patients of epidemic dimension with many patients dying of AKI or its complications [1,2]. There is a large body of literature showing the importance of novel biomarkers of AKI [3–9]. Such markers are central to early diagnosis for timely treatment and potentially cure of AKI. In clinical practice, the currently applied renal biomarkers including serum creatinine, urea and urinary output reflect loss of renal function. These markers delay the diagnosis of AKI by 24–48 h, thereby they may contribute to the lack of specific therapies for AKI despite huge efforts during the last decades.

Neutrophil gelatinase-associated lipocalin (NGAL) is a quite novel and already intensely investigated renal biomarker indicating early structural damage. The source of NGAL and mechanisms of its expression remained elusive until the January issue of Nature Medicine, where a Technical Report was published [10], describing how NGAL expression is monitored by bioluminescence and fluorescence in experimental models of AKI (ischaemia-reperfusion, application of cisplatin or lipid A).

In a first step, a heterozygous diffusion reporter mouse was created that expresses luciferase2 and mCherry (Luc2/mC) under the control of the NGAL promoter. This promoter is responsive to toxic stimuli. Polymerase chain reaction was used to genotype the NGAL-Luc2/mC double fusion reporters. NGAL gene and protein expression in vivo was quantified by photon emission detection and in parallel fusion reporters. NGAL gene and protein expression was used to genotype the NGAL-Luc2/mC double fusion reporters. NGAL gene and protein expression in vivo was quantified by photon emission detection and in parallel fusion reporters. NGAL gene and protein expression was quantified by photon emission detection and in parallel fusion reporters. NGAL gene and protein expression was quantified by photon emission detection and in parallel fusion reporters. NGAL gene and protein expression was quantified by photon emission detection and in parallel fusion reporters. NGAL gene and protein expression was quantified by photon emission detection and in parallel fusion reporters. NGAL gene and protein expression was quantified by photon emission detection and in parallel fusion reporters. NGAL gene and protein expression was quantified by photon emission detection and in parallel fusion reporters. NGAL gene and protein expression was quantified by photon emission detection and in parallel fusion reporters.