Kidney Injury and Regeneration in Zebrafish

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Summary: Renal tubule epithelial cells can regenerate in response to acute injury. Although this process remains poorly understood, it appears to involve the reactivation of pathways that are operative during embryonic kidney formation. A better understanding of renal regeneration may lead to the development of new therapies that can attenuate acute kidney injury or expedite recovery. The zebrafish is being used as a model to understand renal regeneration. In this review, we summarize the current knowledge on zebrafish kidney formation, describe methods for inducing acute injury, and focus on the unique capacity of the zebrafish adult kidney to undergo de novo nephron formation in response to damage.
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The kidney acts as a key organ for waste removal and the homeostatic regulation of electrolytes and water. Central to this role is the nephron, the functional unit of the kidney, which comprises a blood filter (glomerulus) attached to an epithelial tubule that is subdivided into proximal and distal segments. The filtrate produced by the glomerulus is modified extensively by the action of various transporters as it passes through these segments. The glomerulus, containing podocytes, and the proximal tubule, the site of bulk filtrate reabsorption, are common targets of injury and disease. These portions of the nephron have become the focus of recent efforts to assess the regenerative potential of glomerular and proximal tubule cells, with the hope of developing new therapies for acute and chronic kidney damage. The zebrafish model, with its host of embryonic, genetic, and unique regenerative capabilities, has aided in efforts to elucidate the response of the kidney to injury. In this review, we provide an overview of zebrafish embryonic and adult kidney formation and focus on some of the recent contributions this model has made to our understanding of renal regeneration after acute kidney injury (AKI).

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FORMATION AND STRUCTURE OF THE ZEBRAFISH PRONEPHROS

The functional kidney type of the zebrafish embryo is the pronephros, a relatively simple structure composed of two nephrons that are located along the proximal–distal body axis and joined at the cloaca (Fig. 1). Despite its simplicity, the pronephros shares major functions and cell types with nephrons found in other vertebrate kidneys, including the metanephros in mammals.

Pronephros development starts shortly after gastrulation ends, with the formation of nephrogenic tissue from its origin, the intermediate mesoderm (IM). Early molecular markers of the IM include the transcription factor genes *pax2a*, *pax8*, and *lhx1a*[1–3] (Fig. 1). Pax2 and Pax8 act as master regulators of the vertebrate kidney program because zebrafish and mouse embryos deficient in these factors show an early arrest in renal progenitor differentiation, whereas overexpression induces ectopic kidney formation.[4–6] Renal progenitor cells arising from the IM undergo a mesenchymal-to-epithelial transition with the anterior-most cells differentiating into podocytes under the control of the Wilms tumor suppressor-1 (Wt1) transcription factor and the Notch pathway.[7] The remaining renal progenitors give rise to the tubular epithelium of the pronephros and become segmented into two proximal and two distal tubule segments, which are defined by a host of segment-specific marker genes.[8] Retinoic acid signaling and the Mecom/Evi1 transcription factor play key roles in establishing the segmentation pattern of the tubule segments, and the Hnf1b transcription factor is essential for the activation of mature segment-specific genes.[6,8–10] The different portions of the pronephric nephron (from proximal to distal) can be defined as follows: glomerulus, neck segment, proximal convoluted tubule, proximal straight tubule, distal early tubule, and distal late tubule. The vascular supply to the glomerulus forms from sprouts from the overlying dorsal aorta and blood filtration begins approximately 48 hours after fertilization[11] (Fig. 1B).

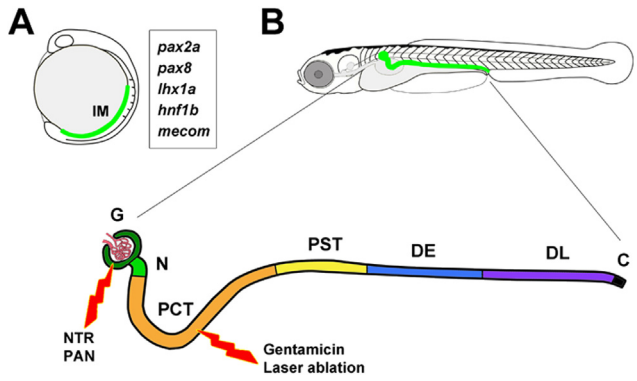

Figure 1. Zebrafish pronephros development and nephron structure. (A) Pronephros formation follows somitogenesis. Nephrogenic transcription factors expressed in the IM include *pax2a*, *pax8*, *lhx1a*, *hnf1b*, and *mecom*. (B) Location of the mature pronephros in a 5-day-old larva. Pronephros segments comprise the glomerulus (G), neck (N), proximal convoluted tubule (PCT), proximal straight tubule (PST), distal early tubule (DE), distal late tubule (DL), and cloaca (C). AKI-like injury to the glomerulus can be induced by NTR-mediated podocyte ablation or PAN injection. Damage to the proximal tubule segments can be generated by gentamicin injection or laser cell ablation.

Cell types in the zebrafish pronephros closely resemble the equivalent cells in the mammalian nephron, regarding cellular structure, function, and gene expression. Podocytes are glomerulus-specific epithelial cells with finger-like extensions, called *foot processes*, that interdigitate with neighboring podocytes and link together by way of protein bridges called *slit diaphragms*.[12] The blood filtrate flows into the urinary space surrounding the podocytes and into the proximal tubule segments, where the bulk of useful molecules such as sugars, salts, and small proteins are reabsorbed. Multiple ion exchangers and molecule transporters are expressed in these segments, many of which are conserved in the proximal tubule segments of the mammalian kidney.[13] The distal portions of the zebrafish pronephric tubules most likely modify the osmolarity of the urine and fine-tune its composition, before it passes out of the embryo via the cloaca.

THE PRONEPHROS AS A MODEL FOR ACUTE KIDNEY DISEASE

Because of the conservation in cell types between zebrafish and mammals, the zebrafish pronephros represents an excellent model for studying kidney development, disease, and repair. Its nearly linear layout allows easy assessment of kidney damage–related phenotypes. Moreover, the rapid development to a fully functioning mature pronephric kidney (by 4 days after fertilization), as well as the almost complete transparency of the zebrafish embryo, permits relatively easy manipulation, observation, and imaging of its components in vivo.

Podocyte Injury

In the past decade, multiple techniques have been developed to induce AKI in the zebrafish pronephros. Hentschel et al[14] showed that puromycin aminonucleoside (PAN) injection into the circulation induces glomerular toxicity similar to that observed in the mouse, including a loss of slit diaphragm integrity and podocyte foot process effacement. The investigators compared the chemical-induced damage with morpholino-mediated knockdown of two genes encoding proteins critical for podocyte functionality, podocin and CD2AP, and obtained comparable results, showing the utility of using PAN to study glomerular injury in the zebrafish.

Functionally, glomerular damage can be assayed by testing the integrity of the glomerular filtration barrier. This assay is performed by injecting a fluorescent molecule (such as dextran) of defined molecular weight into the circulation of embryos with a mature glomerular filtration barrier. Dextran molecules (10-70 kDa) pass through the glomerular filter of healthy control fish within 48 hours and are taken up into endocytic vesicles in the proximal tubules. PAN-induced damage to the glomerulus, however, accelerates the clearance of the dextran, and allows larger-sized molecules (500 kDa), which normally are not filtered, to pass into the nephron and accumulate in the proximal tubules.[15]

Two recent reports have made use of the fast-growing number of transgenic zebrafish lines to genetically induce podocyte injury. Both studies describe temporally inducible podocyte ablation using the bacterial nitroreductase (NTR) strategy to convert a prodrug, metronidazole, into a cytotoxic metabolite. Toxicity to podocytes was induced specifically in larval or adult transgenic zebrafish that express NTR under the control of the *podocin* promoter.[16,17] The investigators of both studies observed podocyte loss, reduced podocyte marker gene expression, and foot process effacement consistent with glomerular injury.

Huang et al[17] observed that after metronidazole washout, proliferating cells were detected in the glomeruli of recovering transgenic fish with a restoration of *nephrin* and *podocin* gene expression, and a re-establishment of normal foot process architecture and barrier function. The source of the regenerated podocytes remains unclear. It is unlikely that new podocytes can arise from mature podocytes because these cells are considered postmitotic.[18] Recent studies in mammals have suggested that podocyte progenitors may exist, at least transiently in juveniles, in Bowman's capsule, the parietal epithelial cell layer that envelops the glomerulus and is in continuity with podocytes.[19–21] Cells of the renin lineage, located in close proximity to the glomerulus, also were described as a potential source of podocyte progenitors in the mouse.[22] Further