A zebrafish model of conditional targeted podocyte ablation and regeneration

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Podocytes are specialized cells that contribute critically to the normal structure and function of the glomerular filtration barrier. Their depletion plays an important role in the pathogenesis of glomerulosclerosis. Here, we report generation of a genetic model of conditional podocyte ablation and regeneration in zebrafish using a bacterial nitroreductase strategy to convert a prodrug, metronidazole, into a cytotoxic metabolite. A transgenic zebrafish line was generated that expresses green fluorescence protein (GFP) and the nitroreductase fusion protein under the control of the *podocin* promoter Tg*(podocin:nitroreductase–GFP)*. Treatment of these transgenic zebrafish with metronidazole results in podocyte apoptosis, a loss of *nephrin* and *podocin* expression, foot process effacement, and a leaky glomerular filtration barrier. Following metronidazole washout, proliferating cells were detected in the glomeruli of recovering transgenic fish with a restoration of nitroreductase–GFP fluorescence, *nephrin* and *podocin* expression, a reestablishment of normal foot process architecture, and glomerular barrier function. Thus, our studies show that zebrafish podocytes are capable of regenerating following depletion, and establish the Tg*(podocin:NTR–GFP)* fish as a new model to study podocyte injury and repair.

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The kidney is a vital organ that performs a number of essential functions, including blood filtration and clearance of endogenous waste products. Podocytes are specialized epithelial cells that contribute critically to the kidney's 'filtration apparatus.' Podocyte dysfunction and/or damage has been associated with both acute and chronic glomerular diseases, including focal segmental glomerulosclerosis, diabetic nephropathy, and HIV nephropathy.[1–6] Podocyte depletion leads to glomerulosclerosis in murine models, and recent studies suggest that short-lived localized insults can trigger a cascade of secondary damage that causes more global injury.[7–9] Understanding how podocytes respond to injury and whether they are capable of regeneration will provide valuable information for the development of new therapies that seek to replace damaged or lost podocytes.[10]

The zebrafish is a widely used vertebrate model organism for the study of developmental mechanisms and disease pathologies for many organs. It combines many advantages including genetic tractability of both forward and reverse genetics, accessibility to observation and manipulation during organogenesis, and a great capability for regeneration after injury.[11–18] Studies from multiple groups have established zebrafish as a useful model system to study kidney development and function.[19–22] Despite the structural simplicity of the zebrafish pronephros, consisting of a single glomerulus in connection with two pronephric tubules, it possesses a glomerular filtration apparatus with a similar complexity to that of the mammalian kidney.[21,23] More recently, it has been utilized as an alternative *in vivo* model for studying kidney injury and regeneration.[16,24–26]

The zebrafish kidney has a remarkable ability to regenerate after injury, and kidney stem/progenitor cells have been identified in adults.[16,26] Similar to a recent report,[27] we have independently established two transgenic zebrafish lines where green fluorescence protein (GFP) and a fusion protein of GFP and the bacterial nitroreductase (NTR) are expressed in podocytes under the control of the *podocin* promoter. In the Tg*(podocin:GFP)* line, the podocytes are fluorescently tagged allowing them to be visualized, isolated, and tracked *in vivo*, whereas the Tg*(podocin:NTR–GFP)* line utilizes bacterial NTR to convert the nontoxic pro-drug metronidazole (Mtz) into a cytotoxic, DNA crosslinking agent that induces cell death.[28,29] Here we report that specific

podocyte ablation and glomerular dysfunction occurs in Tg(*podocin:NTR–GFP*) embryos after treatment with Mtz. Interestingly, following Mtz washout, there is a recovery of glomerular filtration barrier function that is associated with podocyte proliferation in the glomerulus and a restoration of normal podocyte foot process architecture. These findings suggest that zebrafish podocytes are capable of regeneration following depletion and establish the Tg(*podocin:NTR–GFP*) line as a useful model to identify new therapeutic targets involved in the response of podocytes to injury.

RESULTS AND DISCUSSION

Expression of *GFP* and *GFP–NTR* under the control of podocyte-specific *podocin* promoter

We isolated a 3.5-kb DNA fragment located upstream of the *podocin* gene that has previously been found to contain the mouse *podocin* promoter.[30,31] We subsequently ligated GFP and GFP–NTR under the control of this promoter in the Tol2 transposon vector and injected zebrafish embryos with these constructs[32] (Figure 1a). By outcrossing with wild-type fish, we identified four independent founders for both transgenic fish lines, Tg(*podocin:GFP*) and Tg(*podocin:NTR–GFP*), respectively. Embryos from each of the founders displayed identical expression patterns in which GFP was expressed exclusively in the region of the pronephric glomerulus from 60 h after fertilization by fluorescence microscopy (Figure 1b). The founders with the strongest GFP expression were used to collect embryos for study and line maintenance. Consistent with these lines expressing GFP in podocytes, we found that the GFP signal localized to the site of *nephrin* expression (Figure 1c) and they both colocalized with the site of NTR expression in Tg(*podocin:NTR–GFP*) larval fish (Figure 1d).

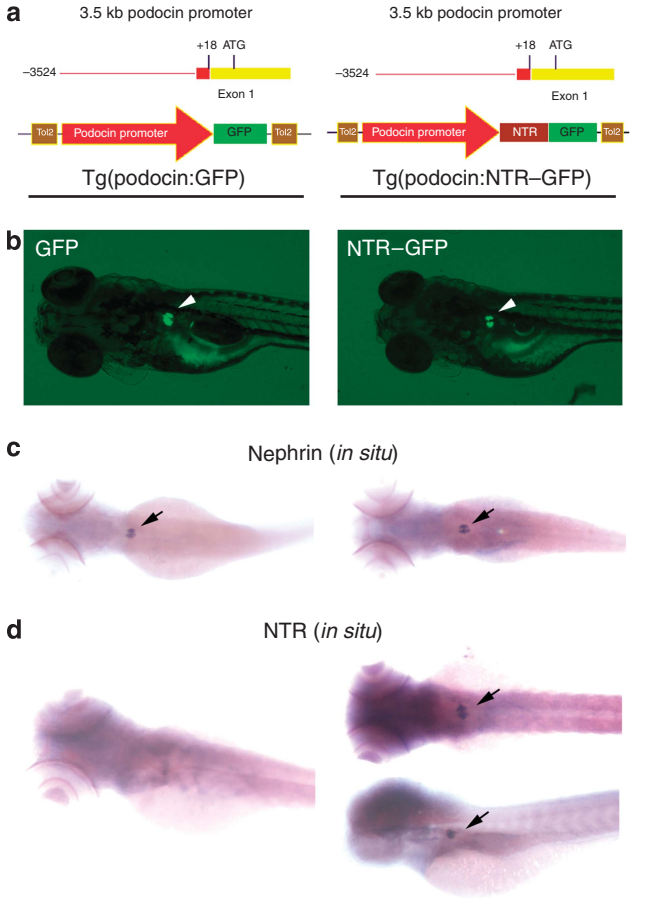

Figure 1 | GFP and NTR–GFP expression under the 3.5-kb *podocin* promoter. (**a**) Constructs used to generate the *podocin*-driven GFP (left panel) and NTR–GFP (right panel) transgenic lines are shown, respectively. GFP, green fluorescence protein; NTR, nitroreductase. (**b**) GFP expression in glomeruli from transgenic fish (left panel, arrow) Tg(*podocin:GFP*) and (right panel, arrow) Tg(*podocin:NTR–GFP*). (**c**) GFP expression (panels in **b**) overlaps with *nephrin* expression in glomeruli from (left panel, arrow) Tg(*podocin:GFP*) and (right panel, arrow) Tg(*podocin:NTR–GFP*) embryos. (**d**) NTR is expressed in glomeruli from (right panel, arrows) Tg(*podocin:NTR–GFP*), but absent in glomeruli from (left panel) Tg(*podocin:GFP*) embryos.

Conditional ablation of podocytes results in a loss of podocyte marker expression and the slit diaphragm, and defective glomerular barrier function

We next determined the conditions under which Mtz will induce conditional ablation of podocytes. Wild-type, Tg(*podocin:GFP*), and Tg(*podocin:NTR–GFP*) larval fish at 70 h after fertilization were incubated with Mtz for 12–48 h at concentrations ranging from 1 to 20 mmol/l. Exposure to Mtz for 12 h resulted in pericardial edema in Tg(*podocin:NTR–GFP*) larval fish, consistent with renal failure (Figure 2a). The extent of pericardia edema was more pronounced with increasing Mtz concentration or prolonged exposure even when low (2 mmol/l) concentration of Mtz was used (data not shown). Concomitant with the presence of pericardial edema, the intensity of the GFP signal in the glomerulus of Mtz-treated Tg(*podocin:NTR–GFP*) larval fish was significantly reduced in a dose-dependent manner (Figure 2b). A robust effect was found when Tg(*podocin:NTR–GFP*) embryos were exposed to Mtz at 4 or 10 mmol/l for 12 h, with ~95% ($n = 41/43$) of the animals showing a marked reduction or loss of GFP fluorescence in the glomerulus (Figure 2b and cB). No effects on GFP signal or the appearance of pericardial edema was observed in Mtz-treated Tg(*podocin:GFP*) embryos for 12 or 48 h (Figure 2a and cA, left panel). When Mtz concentrations of >20 mmol/l were used, we observed nonspecific toxicity, characterized by necrosis of the larva without significant pericardial edema in all groups (Tg(*podocin:NTR–GFP*), Tg(*podocin:GFP*), and wild-type fish; data not shown). Whole-mount *in situ* hybridization showed that the loss of GFP fluorescence induced by Mtz was concomitant with loss of the expression of *nephrin* (Figure 2cD and Figure 4aJ and K) and *podocin* in the glomerulus (Figure 4aF and G). Despite significant edema and reduced expression of GFP/*nephrin*/*podocin* induced by Mtz in Tg(*podocin:NTR–GFP*) animals, we did not detect any abnormalities or change of gene expression in Mtz-treated Tg(*podocin:GFP*) and wild-type larval fish, or in Tg(*podocin:NTR–GFP*) larval fish without Mtz treatment. Ultrastructural examination of the glomerulus from