Pax2a is upregulated in podocytes in a zebrafish injury model

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Objective

There is an ongoing debate about podocyte progenitor cells in glomeruli. To address this question, we used the zebrafish larva as a well-established animal model for kidney research. Zebrafish larvae develop a functional glomerulus with a size-selective filtration barrier already after 56 hours. Since the transcription factor *pax2a* is essential for kidney development and is reported to be upregulated in progenitor cells as well as in patients suffering from chronic kidney disease, we wanted to study the expression of *pax2a* in a podocyte-specific zebrafish injury model.

Methods

We used an eGFP-expressing *pax2a* reporter zebrafish strain and the well-established NTR/MTZ podocyte-injury model. The zebrafish strain mCherry which expresses the fluorophore mCherry and the bacterial nitroreductase (NTR) under the control of the *nphs2* promotor exclusively in podocytes was crossed with the *pax2a* reporter zebrafish. Podocyte injury was induced by addition of the prodrug metronidazole (MTZ, 5 mM or 80 µM) to the E3 medium. The morphology of zebrafish larvae was analyzed by cryosections stained with an antibody against nephrin and *pax2a*. *In vivo* observations were performed by two-photon microscopy (2PM).

Results

In-silico analysis of zebrafish *pax2a* and human PAX2 showed a high homology of 79 % between both species. *Pax2a* expression in zebrafish larvae was found in the hindbrain, spinal cord, otic vesicles and in proximal tubular cells. Interestingly, immunostaining revealed an additional expression of *pax2a* in parietal epithelial cells. We observed that the *pax2a* expression in the glomerulus significantly decreased during nephrogenesis.

The induction of podocyte injury by the application of MTZ led to edema formation as reported previously. Histological sections of the MTZ treated zebrafish larvae showed a strong decrease of the nephrin staining as well as a down-regulation of the mCherry expression. Furthermore, we found an upregulation of the *pax2a* expression in podocytes 6, 12 and 24 hours after induction of the podocyte injury in fixed sections and by *in vivo* 2PM. Moreover, we found that regeneration after the MTZ-treatment resulted in an intensive expression of *pax2a* in parietal epithelial cells as well as in unknown cells on the glomerular tuft demonstrated by immunostaining.

Conclusion

Our experiments reveal that *pax2*a is upregulated in podocytes in a zebrafish injury model as it was reported for patients suffering from focal segmental glomerulosclerosis. Moreover, podocytes start to express *pax2a* during regeneration. Whether this expression is an indication for a regenerative capability of podocytes remains unclear.