The function of heparanase 2 and its connection with kidney function in zebrafish (*Danio rerio*)

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Heparan sulfate glycosaminoglycans are crucial components of the endothelial cell glycocalyx that constitutes together with the endothelial cells, the glomerular basement membrane and the parietal epithelial cells (podocytes) the glomerular filtration barrier in the kidney. The protein heparanase 2 was recently discovered and is speculated to regulate the activity of heparanase 1, a protein well known for cleaving heparan sulfate components of the endothelial glycocalyx. These preliminary results demonstrate that knockdown of heparanase 2 in zebrafish causes an edematous phenotype and proteinuria, probably by disrupting regulation of heparanase 1.

Heparanase 1 (HPSE1) has been studied extensively for its role in various different disease pathologies. Its physiologic function is described as an Endo-beta(1-4)-D-glucuronidase that degrades heparan sulfate polysaccharide side chains of the glycocalyx covering endothelial cells and basement membranes[1]. Several independent groups have shown in diverse animal models and humans that heparanase expression is upregulated in several primary and secondary glomerular proteinuric diseases, including diabetic nephropathy, IgA nephropathy, the heymann nephritis model (MGN) and models for MCD/FSGS, in children affected by steroid sensitive syndrome, and in anti-GBM disease[2-10]. Whereas HPSE1 has been studied extensively, little is known about the more recently discovered heparanase 2 (HPSE2) that was cloned in 2000[11]. HEPSE2 inhibits HEPSE 1 in cell culture experiments, and clinical data indicate HPSE2 expression is markedly elevated in head and neck carcinoma patients, correlating with prolonged time to disease recurrence[12,13]. However, HEPSE2 function remains to be elucidated and has not been studied in an *in vivo model*. In this study, we examined the role of HPSE2 in glomerular and vascular physiology employing a zebrafish model.

Knockdown of HPSE2 gene was performed by morpholino microinjection into 1 to 4 cell stage zebrafish embryos of a transgenic fish line (Tg l-fabp:DBP:eGFP), which produced a green fluorescence vitamin-D binding protein. These fish were compared to injected control morpholino (CTRL) and uninjected wildtype (WT) group of fish. At 72, 96 and 120 hours post-fertilization (hpf); fish were assessed for generalized edema and categorized into four subgroups: P1 (no edema) to P4 (severe edema).

Figure 1. Knockdown of HPSE2 gene causes an edematous phenotype that is not seen in WT and control fish.

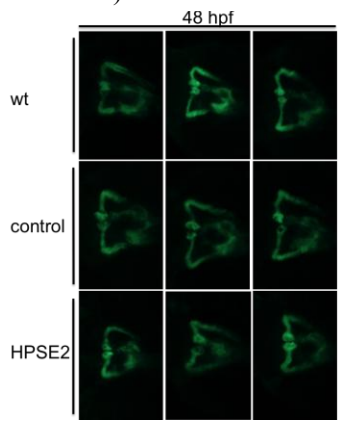

Figure 2. Glomeruli of the pronephros fuse at ~48 hpf as shown in the WT1b fish; this is not influenced by HEPSE2 knockdown.

Injection of HPSE2 morpholino resulted in development of substantial pericardial and yolk sak edema, such that most fish could be classified as P3/4 (Fig 1). In WT1b fish, regular glomerular fusion was seen at 48 hpf (Fig 2) excluding a major disturbance in early kidney development by HEPSE2 knockdown. Edema suggested loss of high molecular weight proteins from the vascular system, and loss of colloidosmotic pressure caused generalized edema. We tested this hypothesis by performing at 96 hpf and 120 hpf the fabp eye assay, which indirectly measures intravascular content of fluorescent 78 kDa high MW protein DBP in the vasculature of fish.

We observed a significant decrease in fluorescence levels in the eye as compared to wild type and control morpholino injected fish (Fig 3). There was greater loss of fluorescence at increasing morpholino

concentrations, suggesting a dose dependence of gene knockdown. These results indicated the glomerular filtration barrier is indeed compromised by HEPSE2 knockdown and that proteinuria occurs as a sequela of that.

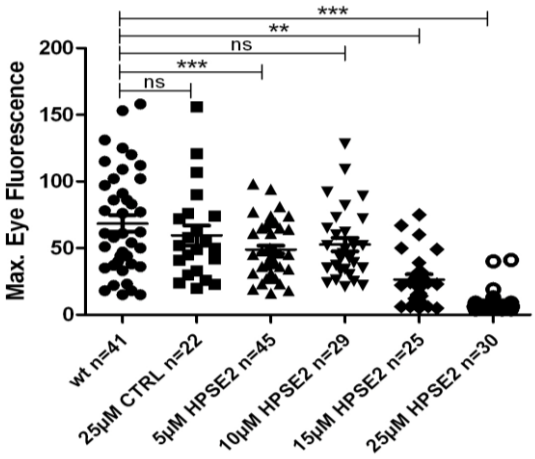

Figure 3. HPSE2 morpholino injected groups had a lower overall fluorescence level as compared to the CTRL and WT groups, indicating loss of high molecular weight plasma proteins into the urine due to damage to the glomerular filtration barrier. Loss of fluorescence was dose-dependent. $^{**}p < 0.01$, $^{***}p < 0.001$; data are mean ± SE.

These data demonstrated a significant role for HPSE2 in the structural integrity of the glomerular filtration barrier. Further experiments are needed to better understand the exact function of this novel gene and will help unravel a novel mechanism involved in causing glomerular injury and acute as well as chronic kidney disease.

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