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The Ebf1 knockout mouse and glomerular maturation

Running title: **Ebf1 in glomerular maturation**

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Abstract

Mice deficient for the transcription factor early B-cell factor 1 (Ebf1), lack mature B lymphocytes, but have additional phenotypes suggesting functions outside the hematopoietic system. Now it is reported that these mice also exhibit quantitative and qualitative developmental renal defects and develop progressive podocyte foot-process effacement. The findings not only suggest that Ebf1 may be pivotal to the transcriptional podocyte network, but also illustrate the importance of distinguishing cell-autonomous and non-autonomous inputs on podocyte maturation and integrity.

Text body

Embryonic renal development and glomerular maturation are intimately linked. During the earliest phases, nephron progenitor cells condense, convert to primitive nephron epithelia, and form patterned early nephron structures called S-shaped bodies. These S-shaped bodies contain a layer of primitive podocytes that initially form a columnar epithelial layer and a flat cell layer comprising future Bowman's capsule.[1] Next, a capillary enters the glomerular cleft and contacts the podocytes to form primitive capillary loop-stage glomeruli. Thereafter, podocytes flatten and form foot processes. This process roughly coincides with the onset of urine production. As urinary filtration increases, additional signs of progressive glomerular maturation can be observed. The capillary loops expand and form a progressively complex network covered by intricate interdigitating podocyte foot processes. The expansion leads to an increasing filtration surface area and a morphological transformation into adult-type mature glomeruli (Fig. 1). Several transcription factors regulate early steps of podocyte differentiation, including Tcf21/Pod1, Lmx1b, Mafb, and Foxc2.[1] In contrast, less is known about the transcriptional mechanisms that coordinate the later phases of glomerular maturation and the maintenance of glomerular integrity.

In this issue of *Kidney Int*, Fretz et al propose a novel role for the early B-cell factor 1 (Ebf1) transcription factor in regulating late glomerular maturation and the maintenance of filtration barrier integrity.[2] Ebf1 belongs to a family of transcription factors containing an atypical zinc-finger DNA-binding domain and a non-basic helix-loop-helix (HLH) dimerization domain. Ebf1 is necessary for the development of mature B lymphocytes, which was first shown almost two decades ago by the absence of these cells in Ebf1 knockout ($Ebf1^{-/-}$) mice.[3] $Ebf1^{-/-}$ mice lack Ebf1 in all cells and thus the presence of other defects comes as no surprise. For instance $Ebf1^{-/-}$ mice exhibit excess apoptosis in the brain striatum.[4] They also have a defect in facial branchiomotor neuronal migration.[5] In addition, the mice are smaller than their littermates and many die before age four weeks for unclear reasons.

Fretz et al. initially studied the role of Ebf1 in osteoblast function in $Ebf1^{-/-}$ mice.[6] They observed elevated serum osteocalcin levels, a non-collagenous protein formed by osteoblasts. While the finding suggested an increased osteocalcin synthesis in $Ebf1^{-/-}$ osteoblasts, the authors paradoxically observed reduced rates of osteocalcin synthesis. Since osteocalcin is cleared by the kidneys,[7] the authors investigated the possibility of renal dysfunction in $Ebf1^{-/-}$ mice. Interestingly, they found a markedly reduced glomerular filtration rate (GFR) in $Ebf1^{-/-}$ mice even after correction for body weight. Furthermore, $Ebf1^{-/-}$ mice developed progressively severe albuminuria. $Ebf1^{-/-}$ kidneys were smaller than those from control littermates, a finding expected on the basis of smaller overall size. However, a detailed histological analysis of these kidneys revealed marked structural abnormalities. The cortex and outer medulla were substantially thinned and immature glomeruli persisted in the outer cortex, while juxtamedullary glomeruli appeared relatively mature. Islands of vimentin-positive cells were observed suggestive of persisting embryonic structures. Also, the size of the glomeruli was reduced in $Ebf1^{-/-}$ mice and the number of capillary loops per glomerulus was reduced. While early

glomeruli detected around the time of birth developed normal foot processes, there was progressive foot process effacement as these mice matured.

Ebf1 expression in normal mouse kidney suggested that Ebf1 was expressed at low levels during renal development and during the first two weeks of life. However, Ebf1 was up-regulated in the more mature kidney (>2 weeks of age), coinciding with the onset of albuminuria in Ebf1$^{-/-}$ mice. Importantly, Ebf1 was expressed in mature glomeruli, suggesting that its expression was dependent on the glomerular differentiation status. Ebf1 was detected in podocytes and several additional neighboring cells. Together, these results suggest a role of Ebf1 in late glomerular maturation and in the maintenance of glomerular integrity.

The phenotype of Ebf1$^{-/-}$ kidneys clearly differs from mice harboring mutations in the core transcription factors governing podocyte differentiation. For instance, the glomerular defects reported in mice lacking the transcription factors Foxc2, Tcf21/Pod1, or Lmx1b occur earlier compared to Ebf1$^{-/-}$ mice and reveal an arrest of glomerular development at the capillary loop stage.[1] The defect observed in Ebf1$^{-/-}$ kidneys is more reminiscent of the kidney phenotypes of mice with defects in the signaling pathways that maintain podocyte integrity. Examples include integrin-linked kinase,[8] or the mammalian target of rapamycin (MTOR) complex,[9] in which initial formation of the podocyte filtration barrier appears to be normal with an insidious onset of proteinuria and foot process effacement. However, in addition to the podocyte defect, Ebf1$^{-/-}$ mice clearly exhibited overall abnormal nephrogenesis. Although Fretz et al. did not assess nephron numbers, their histological analyses suggest that glomerular numbers were reduced. In addition, the fewer capillary loops per glomerulus would lead to a reduced filtration surface. Hence, the low filtration capacity of the Ebf1$^{-/-}$ kidneys may place an inappropriate load on individual glomeruli. This situation could cause single-nephron hyperfiltration and a subsequent progressive glomerular injury.

Fretz et al. [2] conducted molecular analyses indicating that podocytes from Ebf1$^{-/-}$ mice expressed lower levels of Tcf21/Pod1 and Lmx1b, while Mafb and Foxc2 were unchanged. Both Tcf21 and Lmx1b are necessary for glomerular maturation [1] and induced deficiency of Lmx1b in adult podocytes has recently been shown to result in proteinuria and progressive foot process effacement,[10] suggesting that Ebf1 may function to ensure adequate Lmx1b levels in podocytes to maintain podocyte integrity. In addition, Fretz et al. observed reduced production of vascular endothelial growth factor A (VEGFA) in Ebf1$^{-/-}$ podocytes. Podocyte-derived VEGFA maintains glomerular endothelial cell integrity,[11] suggesting that reduced VEGFA expression may also contribute to the Ebf1$^{-/-}$ phenotype.

While the study by Fretz et al. points towards important roles of Ebf1 in renal development and glomerular maturation, several points need to be addressed in future studies. First, the biological basis of the thin renal cortex and persistence of embryonic renal structures of Ebf1$^{-/-}$ kidneys remains unclear. While these phenotypes point to a defect in early kidney development, Ebf1 does not appear to be expressed at high levels in the developing kidney. Thus, extrarenal effects of Ebf1 deficiency could contribute to the developmental renal phenotype. Second, not only podocytes, but also other not yet identified renal cells, express Ebf1 once mice reach age 2 weeks. This coordinated induction of Ebf1 in different cell types of the kidney is surprising and unprecedented. Importantly, the initiation of Ebf1 expression in normal mice coincides with the onset of albuminuria in Ebf1$^{-/-}$ mice. However, whether or not podocyte expression of Ebf1 accounts for the maintenance of glomerular integrity remains unclear. Alternatively, defects of cells outside the podocyte compartment may contribute to the Ebf1$^{-/-}$ phenotype. Finally, the involvement of Ebf1 in osteoblast differentiation has recently been challenged based on the observation that an osteoblast-specific Ebf1 knockout failed to reproduce the bone phenotype observed in global Ebf1$^{-/-}$ mice.[12] In light of these

findings, future studies will need to examine whether or not podocyte-specific deletion of Ebf1 produces similar glomerular defects of global Ebf1$^{-/-}$ mice. Fretz et al. [2] identified the importance of Ebf1 in maintaining a functional kidney. However, future studies will need to resolve whether Ebf1 serves cell-autonomous functions in the podocyte.

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