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Foxc1 and Foxc2 are necessary to maintain glomerular podocytes

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ABSTRACT

Foxc1 and Foxc2 (Foxc1/2) are transcription factors involved in many biological processes. In adult kidneys, expression of Foxc1/2 is confined to the glomerular epithelial cells, i.e., podocytes.

To bypass embryonic lethality of Foxc1/2 null mice, mice ubiquitously expressing inducible-Cre (ROSA26-CreER^{T2}) or mice expressing Cre in podocytes (Nephrin-Cre) were mated with floxed-Foxc1 and floxed-Foxc2 mice. The CreER^{T2} was activated in adult mice by administrations of tamoxifen.

Eight weeks after tamoxifen treatment, ROSA26-CreER^{T2}; Foxc1$^{+/flox}$; Foxc2$^{flox/flox}$ mice developed microalbuminuria, while ROSA26-Cre ER^{T2}; Foxc1$^{flox/flox}$; Foxc2$^{+/flox}$ mice had no microalbuminuria. The kidneys of conditional-Foxc1/2 null mice showed proteinaceous casts, protein reabsorption droplets in tubules and huge vacuoles in podocytes, indicating severe podocyte injury and massive proteinuria. Comparison of gene expression profiles revealed that Foxc1/2 maintain expression of genes necessary for podocyte function such as podocin and Cxcl12. In addition, mice with an innate podocyte-specific deletion of Foxc1/2 by Nephrin-Cre develop similar podocyte injury.

These results demonstrate dose-dependence of Foxc1/2 gene in maintaining the podocyte with a more critical role for Foxc2 than Foxc1 and a critical role of Foxc1/2 in regulating expression of genes that maintain podocyte integrity.

1. Introduction

Foxc1 and Foxc2 (Foxc1/2) belong to subgroup C of the Forkhead-box (FOX) transcription factor superfamily which are involved in development of many organs [1,2]. In humans, mutations of FOXC1 are responsible for the Axenfeld-Rieger syndrome and mutations of FOXC2 underlie the lymphedema-distichiasis syndrome. Mice with the Foxc1 hydrocephalus (ch) mutation have skeletal and ocular anomalies and die perinatally with hydrocephalus due to a lack of calvarial bones [3,4]. Deletion of Foxc2 results in embryonic lethality due to defective cardiovascular development, skeletal, and lymphatic anomalies [5,6]. Foxc1 ch mutants often have abnormal kidney and urinary tract development including duplex urinary system, hydronephrosis and megaureter [7,8]. Foxc2 knockout mice (Foxc2$^{-/-}$) have been reported to have abnormal glomerular capillary tufts [9]. Conditional knockout of Foxc2 in kidneys using Pax2-Cre mice (Pax2-Cre; Foxc2$^{flox/flox}$) develop glomerular cysts with normal glomerular tufts and kidney hypoplasia due to reduced number of nephrons and insufficient elongation of tubules [10].

During kidney development, Foxc1/2 are first expressed in mesenchymal cells surrounding the budding site of the Wolffian duct. Later in development, their expression becomes confined to the podocyte (7, 8, 11, Supplemental Fig. 1). Podocytes are highly differentiated cells and constitute a critical physical filtration barrier within the glomerulus for large molecules such as albumin [13]. Completely overlapping expression and mostly identical DNA binding domain of Foxc1/2 indicate functional redundancy [1,2]. Indeed, mice double heterozygous for Foxc1/2 (Foxc1$^{+/-}$; Foxc2$^{+/-}$) share phenotype with Foxc1$^{-/-}$ mice (duplex urinary system, hydronephrosis and megaureter), with Foxc2$^{-/-}$ mice (abnormal glomerular tufts) and with Pax2-Cre; Foxc2$^{flox/flox}$ mice (glomerular cysts) [9,10,12]. Because all the above strains of mice are embryonically or perinatally lethal, it is difficult to determine the specific role of Foxc1/2 in podocytes. To overcome this limitation, we generated two types of conditional knockout mice for both Foxc1/2, one is knocked out in adulthood by mating with ROSA26-CreER^{T2} mice and the other strain is podocyte-specific knockout created by mating with Nephrin-Cre mice.

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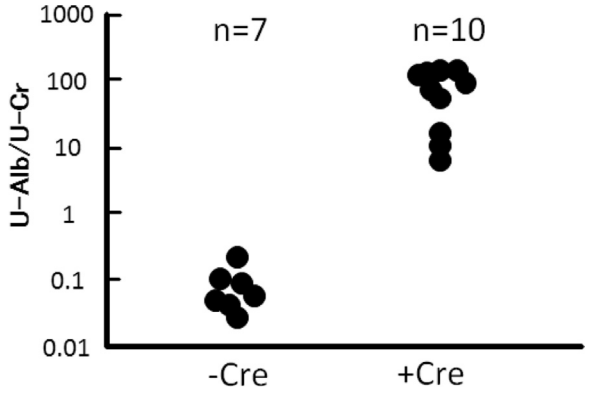

Fig. 1. Development of albuminuria after tamoxifen-treatment in ROSA26-CreER^{T2}; Foxc1$^{flox/flox}$; Foxc2$^{flox/flox}$ mice. Ten ROSA26-CreER^{T2}; Foxc1$^{flox/flox}$; Foxc2$^{flox/flox}$ mice were treated with tamoxifen and urinary albumin/creatinine ratio (mg/mg) was followed for 8 weeks. Each circle represent the highest urinary albumin/creatinine ratio in each mouse. $P < 0.01$ vs. −Cre control mice.

2. Methods

2.1. Mice

All animal protocols were approved by the Animal Experimentation Committee of Tokai University.

Nephrin-Cre, ROSA26-CreER^{T2} mice that express CreER^{T2} ubiquitously, floxed Foxc1 and floxed Foxc2 mice were maintained as described previously [10,12,14–16].

To activate CreER^{T2}, ROSA26-CreER^{T2}: floxed Foxc1/2 mice were injected with tamoxifen i.p. for 3 consecutive days at 8 weeks of age.

2.2. Urinalysis

Concentrations of albumin and creatinine were determined in 24-h urine samples. For mice before weaning, urine samples were analyzed by the polyacrylamide gel electrophoresis.

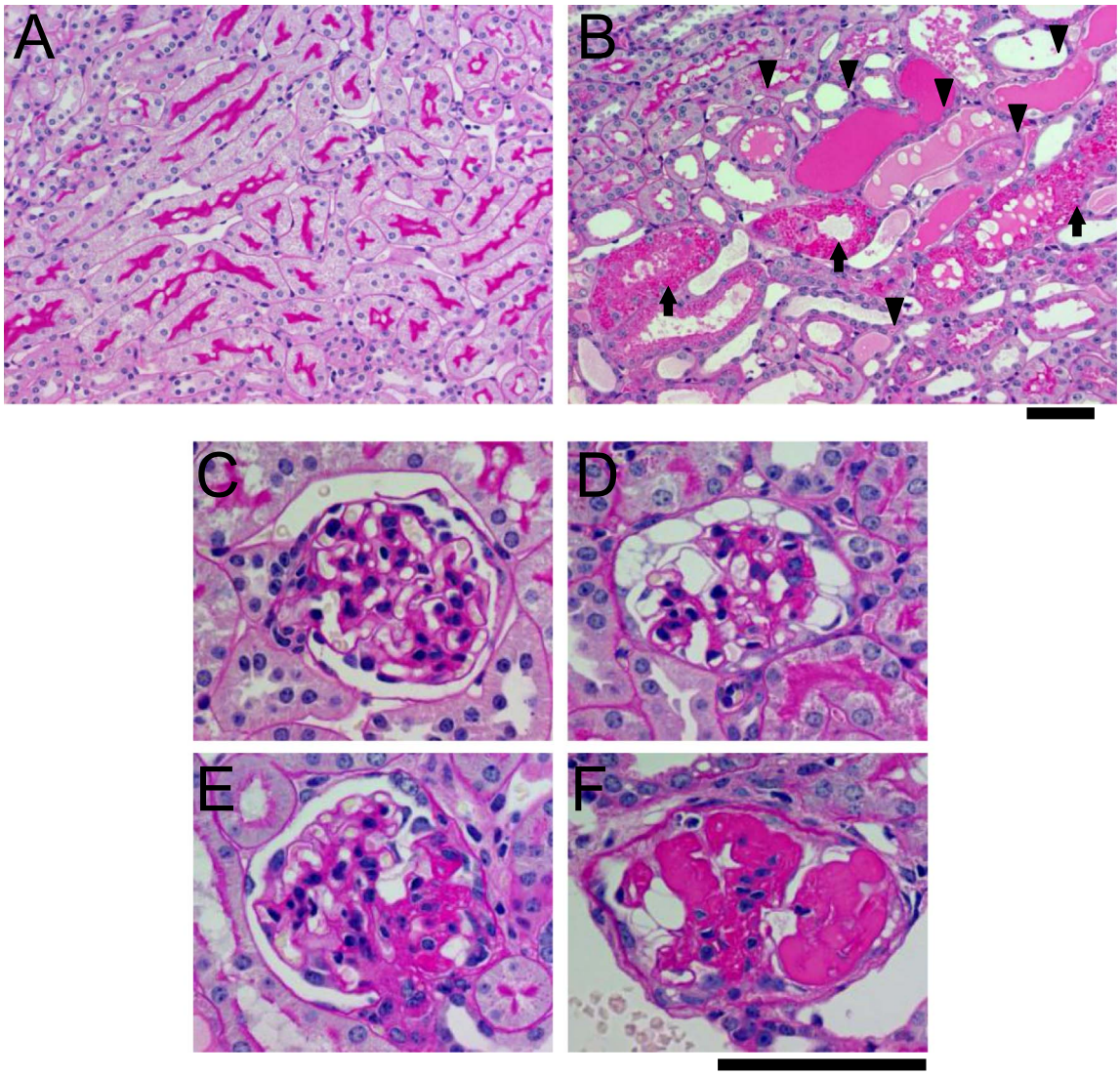

Fig. 2. Renal histology of tamoxifen-treated ROSA26-CreER^{T2}; Foxc1$^{flox/flox}$; Foxc2$^{flox/flox}$ mice. Control mice (Foxc1$^{flox/flox}$; Foxc2$^{flox/flox}$ without CreER^{T2})(A) were normal, whereas CreER^{T2}; Foxc1$^{flox/flox}$; Foxc2$^{flox/flox}$ mice with massive albuminuria (B) showed severe tubular damage with protein reabsorption droplets (arrows) and proteinaceous casts (arrowheads) 8 weeks after tamoxifen-treatment. Control mice showed normal glomerulus (C), whereas CreER^{T2}; Foxc1$^{flox/flox}$; Foxc2$^{flox/flox}$ mice with massive albuminuria often had podocyte vacuolization (D, F) and occasionally segmental (E) and global (F) hyalinosis or sclerosis. Scale bar =50 μm.