Rac1 activation in podocytes induces the spectrum of nephrotic syndrome

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Hyper-activation of Rac1, a small GTPase, in glomerular podocytes has been implicated in the pathogenesis of familial proteinuric kidney diseases. However, the role of Rac1 in acquired nephrotic syndrome is unknown. To gain direct insights into this, we generated a transgenic mouse model expressing a doxycycline-inducible constitutively active form of Rac1 (CA-Rac1) in podocytes. Regardless of the copy number, proteinuria occurred rapidly within five days, and the histology resembled minimal change disease. The degree and severity of proteinuria were dependent on the transgene copy number. Upon doxycycline withdrawal, proteinuria resolved completely (one copy) or nearly completely (two copy). After one month of doxycycline treatment, two-copy mice developed glomerulosclerosis that resembled focal segmental glomerulosclerosis (FSGS) with urinary shedding of transgene-expressing podocytes. p38 MAPK was activated in podocytes upon CA-Rac1 induction while a p38 inhibitor attenuated proteinuria, podocyte loss, and glomerulosclerosis. Mechanistically, activation of Rac1 in cultured mouse podocytes reduced adhesiveness to laminin and induced redistribution of β1 integrin, and both were partially reversed by the p38 inhibitor. Activation of Rac1 in podocytes was also seen in kidney biopsies from patients with minimal change disease and idiopathic FSGS by immunofluorescence while sera from the same patients activated Rac1 in cultured human podocytes. Thus, activation of Rac1 in podocytes causes a spectrum of disease ranging from minimal change disease to FSGS, due to podocyte detachment from the glomerular basement membrane that is partially dependent on p38 MAPK.

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Nephrotic syndrome (NS) is characterized by heavy proteinuria with corresponding hypoalbuminemia and peripheral edema. It is a disorder of the glomerular filtration barrier, a three-layered structure comprising the capillary endothelium, acellular glomerular basement membrane (GBM), and podocytes. Although there is heterogeneity in the disease pathogenesis, podocyte injury is a common end point in all NS forms.[1] Ultrastructurally, podocytes comprise a cell body, primary processes, and actin-rich foot processes that wrap around the glomerular capillaries.[2] These foot processes interdigitate with those from adjacent podocytes, leaving small slits or slit diaphragms that serve as the final size- and charge-selective barrier to the urinary loss of protein.[3] The formation and maintenance of the foot process actin network, and in turn, the integrity of the slit diaphragms, are dependent on many signaling molecules, including the Rho family of small guanosine triphosphatases (Rho-GTPases).[4–6]

Rho-GTPases are commonly referred to as molecular "switches" because they cycle between inactive (guanosine diphosphate bound) and active (GTP bound) states. They are best known for their function as regulators of the actin cytoskeleton.[7] The 3 prototypical members of the Rho GTPase family, namely ras homolog gene family member A (RhoA), Ras-related C3 botulinum toxin substrate 1 (Rac1), and cell division control protein 42 homolog (Cdc42), have each been investigated with respect to their roles in podocyte function and in mediating NS pathogenesis. For example, it has been previously shown that podocyte-specific hyper-activation of RhoA in mice induces proteinuria.[8,9] Furthermore, mice with podocyte-specific ablation of Cdc42 are heavily proteinuric at birth and after disease progression, die from renal failure at 2 weeks of age, whereas the deletion of Rac1 and RhoA does not cause overt phenotypes.[4,6] In injury models, Rac1 deletion in podocytes has been shown to be protective against protamine sulfate–induced acute injury but is deleterious in chronic hypertension–mediated injury.[6]

Genetic studies of familial proteinuric diseases in humans have suggested that Rac1 activation plays an important role in pathogenesis; loss-of-function mutations of the *ARHGAP24* gene, which codes a GTPase-activating

protein that preferentially deactivates Rac1 and Cdc42, have been associated with adult-onset familial focal segmental glomerulosclerosis (FSGS).[10] In addition, loss-of-function mutations of *ARHGDIA*, which codes for a guanine nucleotide dissociation inhibitor that maintains Rho-GTPases in their inactive form, cause congenital or childhood-onset NS,[11–13] as well as Rac1 hyperactivation in cultured podocytes. However, direct evidence that Rac1 hyperactivation induces FSGS or NS is missing. For instance, although Yu *et al.* studied mice that express an inducible constitutively active form of Rac1 (CA-Rac1) in podocytes,[14] the transgene expression was transient for an unknown reason, and the mice exhibited mild transient proteinuria that rapidly resolved. Thus, determining the impact of sustained Rac1 activation in glomerular pathology was not possible.

In the present study, we demonstrate that transgenic mice engineered to express CA-Rac1 in an inducible and podocyte-specific manner[9] develop a range of proteinuria and a spectrum of histologic changes from minimal change disease (MCD) to FSGS, depending on the transgene copy number. Consistent with our previous findings that the effect of Rac1 in podocytes is partly mediated by p38 mitogen-activated protein kinase (MAPK),[15] we found that treatment with a p38 inhibitor attenuates Rac1-induced phenotypes. Finally, we show that Rac1 activation in podocytes is implicated in MCD and idiopathic FSGS in humans.

RESULTS

Active Rac1 in podocytes induces persistent proteinuria in a dose-dependent manner

We generated a transgenic mouse model in which active Rac1 is specifically expressed in podocytes to gain a direct insight into the pathologic role of this critical GTPase (see Materials and Methods). Double transgenic (DTG) mice were treated with doxycycline (Dox) for 5 days, and spot urine samples pre- and post-Dox were analyzed using sodium dodecylsulfate polyacrylamide gel electrophoresis and Coomassie brilliant blue staining. These initial studies revealed the presence of 2 Dox-induced phenotypes: low proteinuric mice, hereafter referred to as low responders, and high proteinuric mice, hereafter referred to as high responders. High responders showed an intense band that corresponded to the molecular size of albumin (67 kDa), indicating that they had heavy proteinuria, whereas low responders developed less severe proteinuria, which was not always apparent by gel staining but was readily detectable by enzyme-linked immunosorbent assay. In contrast, control mice did not show any increase in albuminuria on using either gel staining or enzyme-linked immunosorbent assay. Therefore, we classified DTG mice as high or low responders (Figure 1a, upper panel).

The albumin:creatinine ratio (ACR) of high responders had a magnitude order higher than that of low responders at 5 days after the Dox treatment ($338 \pm 56 \times 10^3$ μg/mg vs. $45 \pm 8 \times 10^3$ μg/mg, n = 4, $P < 0.05$; Figure 1a, lower panel).

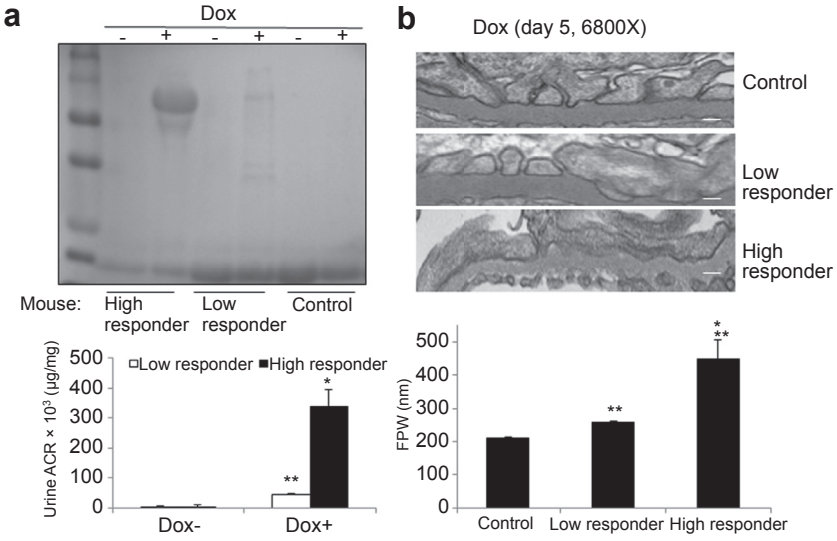

Figure 1 | Podocyte-specific hyperactivation of Ras-related C3 botulinum toxin substrate 1 (Rac1) induces a variable phenotype depending on the dose of active Rac1. (**a**) Detection of doxycycline (Dox)-induced proteinuria (day 5) by Coomassie-stained gel of urine samples separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis. High-responder mice showed an intense band at the molecular weight of albumin (67 kDa), whereas in low-responder mice, proteinuria was barely detectable. Lower panel shows quantification of the urine albumin:creatinine ratio (ACR). *$P < 0.05$ versus low responder, **$P < 0.01$ versus Dox$^-$, n = 4 mice per group. Control mice did not respond to Dox treatment. (**b**) Transmission electron micrographs of the kidneys after 5 days of Dox treatment. Control mice showed intact podocyte foot processes along the glomerular basement membrane (11k ×). Foot process effacement was readily observable in the high-responder mice but was less extensive in the low-responder mice. Bar = 100 μm. Lower panel shows the quantification of the foot process width (FPW), which was inversely correlated with the degree of foot process effacement. *$P < 0.05$ versus low responder, **$P < 0.01$ versus control, n = 3 capillaries from 3 mice per treatment. (Continued)