

TORCing up the Importance of Calcium Signaling

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J Am Soc Nephrol 22: 1391–1393, 2011.
doi: 10.1681/ASN.2011060595

The glomerular filtration barrier is made up of fenestrated endothelium, the endothelial surface layer, glomerular basement membrane, and podocytes forming a slit diaphragm between interdigitating foot processes and the subpodocyte space.¹ Disruption of the filtration barrier results in loss of permselectivity and macromolecules such as albumin in urine. There is evidence to support the relative importance of each component of the filtration barrier in maintaining its integrity.^{2–5} Identification of mutations in a number of genes causing familial FSGS has resulted in significant advances in the understanding of proteinuric kidney disease.^{3,6–10} *In vitro* studies focused on podocyte signaling^{11,12} combined with evidence of increased calcium transients in *TRPC6* mutations seen in familial FSGS^{9,10} support a role for calcium signaling in podocyte dysfunction and disruption of the glomerular filtration barrier.

In this issue of *JASN*, Vassiliadis *et al.*¹³ treated *ex vivo* rat glomeruli with protamine sulfate (PS) to recapitulate minimal change disease in an animal model. They confirmed podocyte foot process effacement and albumin leak from glomeruli occurring as early as 45 minutes after administration of PS. Importantly, using Fluo-4 fluorescence, they showed that intracellular calcium increased in isolated glomeruli almost immediately after treatment with PS. Pretreatment with SKF96365 (a selective inhibitor of receptor-mediated calcium entry), gadolinium (a general ion channel blocker), or EGTA (a Ca^{2+} chelator) prevented the release of albumin from glomeruli. This is significant because it suggests that aberrant increased calcium signaling is an early and, moreover, a necessary event in the development of albumin leakage.

NFAT-dependent transcription is induced by *TRPC6* through calcineurin in both podocytes¹² and cardiomyocytes,¹⁴ with *TRPC6* and NFAT fulfilling a pathogenic posi-

tive-feedback loop in cardiac hypertrophy.¹⁵ Calcineurin also directly dephosphorylates the actin-stabilizing protein synaptopodin, leaving it prone to Cathepsin L (CATL)-mediated degradation. This leads to NFAT-independent alterations in actin polymerization and proteinuria.¹⁶ Vassiliadis *et al.*¹³ tested the hypothesis that calcium-mediated proteinuria and foot process effacement in the PS-treated glomeruli are mediated through the calcineurin/synaptopodin axis. This was confirmed by the observation of decreased expression of full-length (110 kD) synaptopodin, which was prevented by pretreatment with E64, a CATL inhibitor. Inhibition of synaptopodin cleavage by CATL directly with E64 or indirectly with calcineurin inhibitor FK506 or cyclosporine reduced albumin leak from the pretreated glomeruli.

The mammalian target of rapamycin (mTOR) signaling in the glomerulus has been the focus of intense investigation recently.^{17,18} mTOR is a widely expressed serine/threonine kinase of 289 kDa that exists as part of two multimeric complexes: TORC1 and TORC2. TORC1 includes the key scaffolding protein Raptor (regulatory associated protein of mTOR), whereas TORC2 includes Rictor (rapamycin-insensitive companion of mTOR).¹⁹ As the nomenclature suggests, rapamycin binding to FKBP12 (FK-binding protein of 12 kDa) acutely inhibits mTORC1, whereas mTORC2 is relatively insensitive to acute effects of rapamycin.^{20,21} mTORC1 regulates proliferation and inhibits autophagy, thereby controlling cell size,²¹ whereas mTORC2 controls cell survival with phosphorylation of the AKT and protein kinase C (PKC).²⁰ The balance of activity of the TOR signaling, which is described in a number of reviews, and the context in which it happens will determine the net effect on the cell.^{20,22}

Vassiliadis *et al.*¹³ investigated whether mTOR signaling was involved in the calcium-dependent albumin leakage in the PS injury model. Indeed, rapamycin ameliorates the effects of PS administration in a dosage-dependent manner. They carefully investigated the downstream components of the mTORC1 and mTORC2 pathways and observed that phosphorylation of the mTORC1 targets 4E-BP1 and 70S6K was not enhanced by PS treatment, indicating the PS-induced changes did not require mTORC1 activation. In a similar manner, PKC α and serine 473 of Akt, the downstream targets of mTORC2, were also investigated. Phosphorylation of Akt at serine 473 is activated by PS treatment, whereas PKC α is unchanged. Accordingly, rapamycin treatment dramatically inhibits Akt Ser473 phosphorylation, whereas it only partially attenuates phosphorylation of mTORC1 targets 4E-BP1 and 70S6K. Acute inhibition of the mTORC2 complex by rapamycin is discordant with previous reports²⁰; however, the authors cite the high dosage used in this study as a possible explanation.

It is known that Akt is also activated by an alternate path-

Published online ahead of print. Publication date available at www.jasn.org.

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way: phosphorylation at Threonine 308 mediated by phosphoinositide-3-kinase (PI3K).²³ The authors show that Akt activation is PI3K independent: There is no phosphorylation at threonine 308 and the protein leak is ameliorated by administration of curcumin (an mTORC2/Akt inhibitor), whereas administration of PI3K inhibitors (Wortmannin or LY-294002) has no effect on proteinuria. Finally, the authors show that mTORC2/Akt signaling is indeed calcium dependent: The Akt phosphorylation at Ser473 is blocked when the PS-exposed glomeruli are pretreated with SKF96365, the receptor-operated calcium blocker.

This careful study underlines the importance of calcium signaling as an early mediator of glomerular injury. It also implicates calcium signaling changes as an event that triggers calcineurin-dependent Cathepsin L-mediated synaptopodin cleavage, thereby disrupting the glomerular filtration barrier. Perhaps even more interesting is the novel pathway linking increased intracellular calcium with mTORC2 activation, Akt phosphorylation, podocyte effacement, and proteinuria. Although a number of studies showed a protective effect of rapamycin treatment in animal models of glomerular injury,^{24–27} the human experience has been different, with some patients developing irreversible proteinuria when treated with this drug. It is likely that mTOR signaling is heterogeneous and the context in which it is targeted could yield protective or harmful effects.²⁰

A concern that has been raised with mTORC1-selective inhibitors, such as rapamycin, is that selective interruption of the negative-feedback loop through 70S6K, PI3K, and Akt might leave downstream targets of mTORC1 hyperactivated with potential harmful effects.²⁸ In this study, the high dosage of rapamycin used affects mTORC2 rather than mTORC1, although it is unlikely that an equivalently high dosage could be tolerated in humans. However, mTOR kinase inhibitors that target both TORC complexes and dual PI3K are being studied in the oncology field. At least six of these agents are being studied in phase I or phase II National Institutes of Health-sponsored clinical trials.²⁹ It will be interesting to see whether glomerular disease is reported for patients receiving these compounds. Recent studies showed that although abnormally increased mTORC1 activation is a critical event in the development of diabetic nephropathy,¹⁸ genetic modulation of TORC by podocyte-specific deletion of either Raptor or Rictor results in proteinuria, whereas heterozygous deletion of Raptor has a protective effect in an animal model of diabetic nephropathy.¹⁷

The effect of rapamycin administration in animal models of glomerular disease has been incongruous with proteinuria sometimes seen in humans. The studies by Vassiliadis *et al.*¹³ allied to the aforementioned targeted genetic deletion studies,^{17,18} and the ongoing clinical study of small-molecule mTOR kinase inhibitors for treatment of solid tumors advance our understanding of TOR signaling. This knowledge can be applied to identify new therapeutic targets for the treatment of glomerular disease.

ACKNOWLEDGMENTS

M.P.W. is supported by funding from National Institutes of Health grant 5R01DK74748-5 and the Mandel Foundation.

DISCLOSURES

None.

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See related article, "Calcium Mediates Glomerular Filtration through Calcineurin and mTORC2/Akt Signaling," on pages 1453–1461.

Fibrosis, Regeneration, and Aging: Playing Chess with Evolution

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J Am Soc Nephrol 22: 1393–1396, 2011.
doi: 10.1681/ASN.2011060603

In this issue of *JASN*, Iyoda *et al.*¹ report that administration of nilotinib, a second-generation inhibitor of cellular Abelson (c-ABL) kinase, starting at 2 weeks after subtotal nephrectomy decreases glomerular hypertrophy, glomerular sclerosis, tubulointerstitial inflammation, and fibrosis in remnant kidneys and improves renal function and survival. These results are consistent with those obtained with Imatinib, the c-ABL inhibitor prototype, in models of glomerular disease and unilateral ureteral obstruction (UUO). Nilotinib is also effective in animal models of pulmonary² and hepatic fibrosis.^{3,4} Iyoda *et al.* suggest that nilotinib may prove useful in limiting the progression of chronic kidney disease (CKD) to ESRD.

Imatinib was approved by the Food and Drug Administration to treat Philadelphia chromosome–positive chronic myeloid leukemia (CML) in 2001 and has been remarkably successful.⁵ The Philadelphia chromosome contains a constitutively active oncogenic tyrosine kinase (BCR-ABL) created through translocation of a section of human chromosome 9 containing c-ABL with a specific breakpoint cluster region (BCR) on chromosome 22. The second-generation c-ABL inhibitors nilotinib and dasatinib were approved in 2006 for patients who have CML and are experiencing relapse or are intolerant to imatinib. Recent clinical trials comparing nilotinib and dasatinib with imatinib showed superiority of nilotinib and dasatinib compared with imatinib over the first year.^{5–7}

Imatinib, nilotinib, and dasatinib are competitive inhibitors of various tyrosine kinases with different pharmacologic profiles.^{8,9} Imatinib inhibits discoidin domain receptor 1 (DDR) > PDGF receptor α/β (PDGFR α/β) > stem cell factor (SCF) receptor c-KIT > DDR2 > BCR-ABL1 > colony-stimulating factor 1 receptor (CSF1R). Nilotinib, structurally related to imatinib, and dasatinib, a dual SRC and ABL inhibitor and structurally unrelated to imatinib, are 20 and 300 times more potent against BCR-ABL, respectively. Nilotinib is more potent against DDR1 and 2 and as potent against PDGFR α/β , c-KIT, and CSF1R. Dasatinib is also active

Published online ahead of print. Publication date available at www.jasn.org.

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