HHS Public Access

Author manuscript

Kidney Int. Author manuscript; available in PMC 2013 July 28.

Published in final edited form as:

Kidney Int. 2012 September ; 82(5): 507–509. doi:10.1038/ki.2012.154.

PI3 kinase and TGF-β in glomerular nephropathy: what comes first?

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Abstract

TGF-β and PI3K isoforms contribute to glomerular disease. In this issue of Kidney International, Finer and colleagues define a temporal and selective role for the p110γ catalytic isoform of PI3K, normally expressed by hematopoietic cells, and TGF-β in adriamycin-mediated glomerular injury. Early ectopic upregulation of p110γ by podocytes drives initial injury and proteinuria, while late upregulation of TGF-β drives fibrogenesis. Thus, proteinuria and renal fibrogenesis involve distinct signaling activated by p110γ and TGF-β, respectively.

Transforming growth factor (TGF)-β is a well-established pro-fibrotic cytokine that is activated and/or upregulated in the course of glomerular injury. Both mesangial cells and podocytes in the glomerulus of the kidney respond to TGF-β by activating pro-fibrotic and pro-apoptotic signaling. TGF-β exerts its functions via activation of the serine/threonine kinase TGF-β receptors I (TβRI) and II (TβRII)[1]. Binding of TGF-β to the constitutively active TβRII leads to phosphorylation and activation of TβRI. The activated TβRI initiates Smad-dependent and -independent signaling. In Smad-dependent signaling, serine phosphorylation of Smad2 and/or Smad3 occurs, thus resulting in their association with Smad4, translocation to the nucleus, and transcription of target genes. In Smad-independent signaling, adaptor proteins can associate with TβRI or TβRII promoting the activation of pathways such as MAPK, PP2A, JNK, and p38 MAPK. Some of these pathways regulate Smad activation, while others induce responses unrelated to transcription, including cell growth and migration. Both Smad-dependent and -independent pathways have been implicated in the development and progression of fibrosis, and inhibition of TGF-β ameliorates glomerulosclerosis following injury. In addition to the kinases indicated above, TGF-β can affect cell function by cross-talking with phosphatidylinositol-3-kinase (PI3K). The observation that PI3K is a key downstream player of TGF-β-mediated signaling comes from studies showing that this kinase is required for TGF-β-dependent upregulation of lysyl oxidases and monocyte migration, as well as epithelial-to-mesenchymal transition[2, 3]. Finally, high-glucose-mediated activation of PI3K is a key step in promoting TGF-β synthesis[4], clearly indicating a requirement of PI3K for both TGF-β synthesis and function.

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Conflict of interest AP declares no conflict of interest

The PI3K family of enzymes comprises eight catalytic isoforms subdivided into three classes, namely class I, IA, and IB. The catalytic PI3K isoforms can be regulated by receptor tyrosine kinases and/or G protein-coupled receptors and activate AKT, serum/glucocorticoid regulated kinase (SGK), phosphoinositide-dependent kinase 1 (PDK1), mammalian target of rapamycin (mTOR), and several other pathways that control a variety of cellular processes including proliferation, growth, apoptosis, migration, and metabolism[5]. In addition to kinase-mediated signaling, some PI3K isoforms also serve as scaffolds with kinase-independent signaling functions. The class IB catalytic subunit (p110γ) associates with the p101, p87 or p84 regulatory subunits and it is usually activated by G protein-coupled receptors. This catalytic subunit is highly expressed in hematopoietic cells and plays a major role in regulating pro-inflammatory signaling. Recently, a role of p110γ in non-hematopoietic cells in the control of insulin resistance and β-adrenergic receptor function was suggested[6, 7]. However, whether glomerular cells express this catalytic isoform, whether this isoform contributes to glomerular injury, and whether p110γ is a downstream effector of TGF-β has not been investigated.

In this issue of Kidney International, Finer and colleagues[8] describe selective and temporal roles of p110γ and TGF-β in regulating adriamycin-mediated glomerular injury. Adriamycin is a well-established rodent model of chronic kidney disease. Adriamycin leads to podocyte injury followed by glomerulosclerosis, tubulointerstitial fibrosis and end stage kidney disease. BALB/C mice are susceptible to adriamycin-mediated injury and develop classical focal segmental glomerulosclerosis[9]. However, two problems related to the use of BALB/C mice are that following adriamycin treatment they develop a diffuse and aggressive pattern of injury and they often recover within 8 weeks after injury. Finer and colleagues provide evidence that 129×1/SvJ mice represent a better model to study adriamycin nephropathy, as they develop proteinuria, nephrotic syndrome and glomerular and tubulointerstitial accumulation of extracellular matrix components sequentially after ADR administration[8]. Thus, this model allows a better sequential and temporal analysis of pathways activated in the early versus late stages of renal injury.

Based on the finding that increased expression of TGF-β has been observed in many models of glomerular injury, including adriamycin nephropathy, the authors analyze the effect of a soluble TβRII antagonist on both adriamycin-mediated fibrosis and proteinuria. The authors provide evidence that inhibiting TGF-β reduces fibrosis, without affecting proteinuria[8]. This surprising result suggests that pathways classically activated by TGF-β do not contribute to the damage of the glomerular filtration barrier in this injury model. Importantly, selective inhibition of the p110γ isozyme of PI3K with AS605240 reduces adriamycin-mediated proteinuria and injury, suggesting that this kinase contributes to podocyte injury. This is an interesting finding, as the p110γ isoform is particularly highly expressed in lymphoid cells, with low-to-modest expression in other organs. The contribution of p110γ in adriamycin nephropathy is corroborated by the finding that *in vivo* upregulation of p110γ by podocytes coincides with concomitant downregulation of nephrin immediately after injury[8]. Treatment with AS605240 prevents nephrin downregulation *in vivo* as well as cytoskeletal disorganization and podocyte apoptosis *in vitro*, clearly suggesting that upregulation of

p110γ by podocytes plays a deleterious role by contributing to loss of the glomerular filtration barrier and proteinuria.

The findings of Finer and colleagues suggest that adriamycin regulates both podocyte (i.e. p110γ) and non-podocyte (i.e. TGF-β) targets. In addition to podocytes, mesangial cells are a target of adriamycin both *in vivo* and *in vitro*. In this context, it has been shown that adriamycin leads to *in vivo* mesangiolysis as early as 48 hours of treatment and increases mesangial production of reactive oxygen species and matrix deposition[10]. Reactive oxygen species can upregulate the expression of various growth factors and cytokines, including TGF-β. Thus, adriamycin might contribute to the fibrotic phenotype predominantly by targeting the mesangial cell/TGF-β axis, and to proteinuria predominantly by targeting the podocyte/p110γ axis (Figure 1).

In an attempt to determine what pathway is activated first in the course of adriamycin-mediated injury, Finer and colleagues analyzed TGF-β expression and the contribution of p110γ at early stages of injury. They provide evidence that proteinuria is evident as early as 3 days post adriamycin injection; however the levels of TGF-β mRNA increase at later stages after injury[8]. Thus, proteinuria precedes TGF-β synthesis. Interestingly, early treatment with AS605240 prevents adriamycin-mediated proteinuria as well as matrix deposition and synthesis, suggesting that either p110γ has a direct pro-fibrotic role or that it might control TGF-β synthesis or function. The latter possibility is supported by the fact that, although PI3K may contribute to TGF-β-mediated pro-fibrotic signaling, either p110γ activity or the resulting downstream proteinuria controls TGF-β production. Previous studies by the authors showed that PI3K plays a direct role in TGF- β-stimulated mesangial cell fibrogenic signaling. Using a general inhibitor of PI3K (LY294002) and the selective p110γ inhibitor AS605240, the authors elegantly showed that only LY294002, but not AS605240, prevents TGF-β-mediated collagen synthesis in renal cells[8]. This result allowed the authors to conclude that p110γ does not directly control matrix deposition nor it is a downstream mediator of TGF-β signaling. Conversely, a PI3K isoform other than p110γ contributes to mesangial cell fibrogenesis.

A possible explanation for the beneficial effects of AS605240 is that its anti-fibrotic action might be due to its ability to negatively regulate TGF-β production. Consistent with this hypothesis, adriamycin-mediated TGF-β production *in vivo* is blunted by AS605240 treatment[8]. However, given the inhibition of proteinuria by AS605240, and the time delay between p110γ-mediated podocyte injury (3 days after injury) and peak of TGF-β production (12–14 days after injury), it is possible, even likely, that p110γ does not directly regulate TGF-β, but that such production is in some way mediated by podocyte injury or subsequent proteinuria (Figure 1).

Although this study clearly shows that selective isozymes of the PI3K family control podocyte function and stability, a shortcoming is that the authors only analyzed one model of glomerular injury, thus making it difficult to determine whether upregulation of p110γ is restricted to adriamycin-mediated injury or whether it is more generally observed in other models of podocyte injury. It would also be interesting to determine whether and when

activation or upregulation of this selective isozyme becomes evident in human renal disease, as it would make p110γ a desirable and selective target for renal injury.

Despite these shortcomings, the novelty of this paper can be summarized as following; 1) the authors provide evidence that the 129×1/SvJ mouse is susceptible to adriamycin-mediated nephropathy and suggest that this model can be used to study the temporal changes that occur in focal segmental glomerulosclerosis; 2) this is the first demonstration that p110γ, an isoform of PI3K that is expressed by lymphatic cells, is upregulated in injured podocytes both *in vivo* and *in vitro*; 3) this PI3K isoform contributes to podocyte injury by downregulating nephrin expression and altering the cytoskeleton; 4) selective blockade of p110γ ameliorates early proteinuria before upregulation of TGF-β is evident; and 5) it provides a selective function for TGF-β versus p110γ in driving adriamycin-mediated injury. Thus, this study shows that proteinuria and renal fibrogenesis involve distinct cell types and signaling mechanisms (Figure 1), and suggest that inhibiting p110γ might have a better overall beneficial effect than traditional anti-TGF-β therapy early in the disease course.

Acknowledgments

These studies were in part supported by a Merit Review from the Department of Veterans Affairs; the NIH grants 2P01DK065123; and the O'Brien P30DK79341.

References

1. Massague J. How cells read TGF-beta signals. Nat Rev Mol Cell Biol. 2000; 1:169–178. [PubMed: 11252892]
2. Voloshenyuk TG, Landesman ES, Khoutorova E, et al. Induction of cardiac fibroblast lysyl oxidase by TGF-beta1 requires PI3K/Akt, Smad3, and MAPK signaling. Cytokine. 2011; 55:90–97. [PubMed: 21498085]
3. Kattla JJ, Carew RM, Heljic M, et al. Protein kinase B/Akt activity is involved in renal TGF-beta1-driven epithelial-mesenchymal transition in vitro and in vivo. Am J Physiol Renal Physiol. 2008; 295:F215–225. [PubMed: 18495798]
4. Hao J, Liu S, Zhao S, et al. PI3K/Akt pathway mediates high glucose-induced lipogenesis and extracellular matrix accumulation in HKC cells through regulation of SREBP-1 and TGF-beta1. Histochem Cell Biol. 2011; 135:173–181. [PubMed: 21240525]
5. Blajecka K, Borgstrom A, Arcaro A. Phosphatidylinositol 3-kinase isoforms as novel drug targets. Curr Drug Targets. 2011; 12:1056–1081. [PubMed: 21291386]
6. Becattini B, Marone R, Zani F, et al. PI3Kgamma within a nonhematopoietic cell type negatively regulates diet-induced thermogenesis and promotes obesity and insulin resistance. Proc Natl Acad Sci U S A. 2011; 108:E854–863. [PubMed: 21949398]
7. Vasudevan NT, Mohan ML, Gupta MK, et al. Inhibition of protein phosphatase 2A activity by PI3Kgamma regulates beta-adrenergic receptor function. Mol Cell. 2011; 41:636–648. [PubMed: 21419339]
8. Finer G, Schnaper HW, Kanwar YS, Liang X, Lin HY, Hayashida T. Divergent roles of Smad3 and PI3-kinase in murine adriamycin nephropathy indicate distinct mechanisms of proteinuria and fibrogenesis. Kidney International. 2012 Accepted.
9. Chen A, Sheu LF, Ho YS, et al. Experimental focal segmental glomerulosclerosis in mice. Nephron. 1998; 78:440–452. [PubMed: 9578071]
10. Chen X, Moeckel G, Morrow JD, et al. Lack of integrin alpha1beta1 leads to severe glomerulosclerosis after glomerular injury. Am J Pathol. 2004; 165:617–630. [PubMed: 15277235]

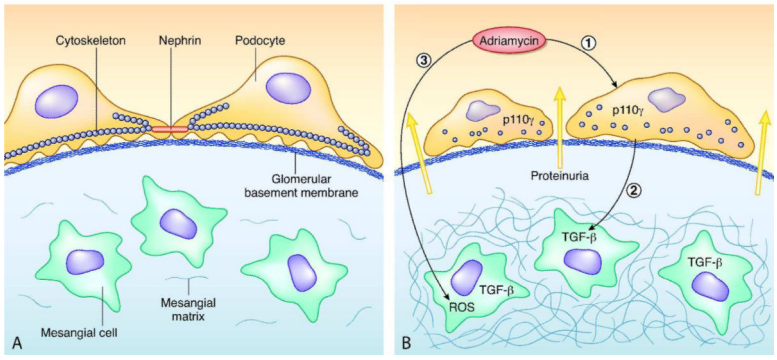

Figure 1.

Schematic representation of podocytes and mesangial cells in healthy conditions (**A**) and following exposure to adriamycin (**B**). Adriamycin leads to upregulation of the p110γ catalytic isoform of PI3K in podocytes with concomitant loss of nephrin, cytoskeletal disorganization and apoptosis (1). This, in turns, leads to proteinuria, more injury and increased expression of TGF-β by mesangial cells with consequent fibrosis (2). Adriamycin can also directly affect mesangial cells by promoting proliferation and generation of pro-fibrotic reactive oxygen species (3).