Kindlin-2: A New Player in Renal Fibrogenesis

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The founding member of the Kindlin family of proteins, Kindlin-1, received its name when it was shown that a loss-of-function mutation in its gene gives rise to a rare, autosomal-recessive disease of the skin called Kindler syndrome (poikilodermia with blisters and keratosis). Kindlin-1 is a constituent of focal adhesions in keratinocytes and interacts with β-integrins. The Kindlin family of focal adhesion proteins (also named FERM family homologs, FERMT) consists of three evolutionarily conserved members—Kindlin-1, -2, and -3—with up to 60% sequence homology. Among the three Kindlins, Kindlin-2 has the broadest expressions in organs and tissues and is highly expressed in kidney. Its primary role was thus far thought to be the interaction with β-integrins (mainly β1- and β3-integrins) and contributions to outside-in as well as inside-out integrin signaling.

In this issue of JASN, Wei and coworkers convincingly show a novel, potentially important integrin-independent function of Kindlin-2 in the kidney, namely interaction with the TGF-β type I receptor (TBR1) and with its major signaling substrate, smad3. Moreover, these investigators demonstrate in vivo that Kindlin-2 positively regulates TGF-β/smad3-dependent profibrogenic signals independent of its interactions with β-integrins. In a commonly used rodent model of TGF-β–mediated renal fibrosis, unilaterally obstructive nephropathy in mice, knock-down of Kindlin-2 reduces interstitial fibrosis. Taken together, Kindlin-2 serves as a TBR1/smad3 adapter protein, directs and augments TGF-β signals toward the smad3 pathway, and amplifies profibrogenic effects of this cytokine in tubular cells in vitro and in a mouse model of renal fibrogenesis in vivo.

The experiments by Wei et al. show in considerable detail how Kindlin-2 interacts with the TBR1 receptor: It binds to the receptor with its FERM (4.1 protein, ezrin, radixin, moesin) domain (consisting of the F1, F2, and F3 subdomains). Interestingly, the F3 subdomain in the Kindlins is also an important domain for their binding to β-integrins. This subdomain is located near the N-terminus, which was determined by Wei et al. as the site of interaction of Kindlin-2 with smad3. Hence, it appears to be likely that at a given time Kindlin-2 interacts with either β-integrins or TBR1/smad3, but not both. Indeed, this notion is consistent with the finding by Wei and colleagues that augmentation of smad3 signaling by Kindlin-2 is independent of β-integrins.

The expression and levels of Kindlin-2 are upregulated in unilateral obstructive nephropathy in mice and in renal fibrosis in human kidney biopsy tissue together with TGF-β and smad3. It appears that TGF-β upregulates expression of Kindlin-2, likely by transcriptional activation. This is inferred from the finding that Kindlin-2 is induced by TGF-β and from findings in podocytes where TGF-β increases Kindlin-2 levels. Hence, TGF-β induces its own profibrogenic signaling activator. It is unknown whether Kindlin-2 raises TGF-β levels. Some findings in the current experiments by Wei and his coworkers appear to support the notion that Kindlin-2 raises TGF-β protein levels: In vivo knockdown of Kindlin-2 in mice with obstructive nephropathy reduces TGF-β levels in kidney (Figure 8, C and G, in Wei and colleagues’ article). Given the known transcriptional autoinduction of TGF-β, the lowered levels upon Kindlin-2 knockdown may be a result of reduced smad3 signal activity toward TGF-β1 gene transcription. Alternatively, Kindlin-2 may act through integrin signaling to regulate TGF-β levels.

TGF-β induces its profibrogenic effects in the kidney mainly through smad3 signaling rather than smad2. At least in some settings, smad2 may even reduce TGFβ1–smad3–driven renal fibrogenesis. One important question is whether the adapter protein Kindlin-2 preferentially or exclusively augments smad3 signals downstream of the TGF-β receptor or also binds to smad2 and facilitates its signals. This question is not addressed in great detail in the studies by Wei and his colleagues, but some of their data provide circumstantial evidence. In Figure 3, A and B, in their article, these investigators show data from co-immunoprecipitation experiments. The findings indicate that Kindlin-2 also associates with smad2 but perhaps quantitatively less or more weakly compared with smad3. There also appears to be less or weaker co-localization of Kindlin-2 with smad2 than with smad3, as may be deduced from immunofluorescence staining (Figure 3C in their article). This latter figure also indicates lesser nuclear co-localization of Kindlin-2 with smad2 upon TGF-β compared with smad3. Thus, although not fully proven, circumstantial evidence suggests that Kindlin-2 preferably augments signaling toward the profibrogenic smad3 pathway in the kidney. Another TBR1 adapter
protein, SARA (smad anchor for receptor activation), appears to preferably interact with smad2 and to direct TGF-β activity toward the smad2 pathway.7,8 Taken together, a model takes shape where it is the adapters, SARA and Kindlin-2, that determine TGF-β effects in given cell contexts as profibrogenic (Kindlin-2) or “not-so-much” profibrogenic (SARA). Validation of such a model will require additional studies; if verified, it may help to design more targeted antifibrogenic therapies without inhibiting all effects of TGF-β.

Kindlin-2 is unique among the Kindlin family of proteins in that it possesses a nuclear localization sequence and cell- and context-dependently translocates into the nucleus.9,10 In human kidney (HK) cells, which were used in the in vitro experiments by Wei and associates, there also appears to be nuclear localization of Kindlin-2 at baseline as well as upon stimulation with TGF-β. After incubation with TGF-β, it seems that Kindlin-2 co-localizes with smad3 in the nucleus. It remains unknown what nuclear function, if any, Kindlin-2 may have perhaps less likely given the nuclear presence in HK cells at baseline before exposure of the cells to TGF-β. The data in Wei and colleagues’ article also do not clearly indicate whether nuclear translocation of Kindlin-2 occurs also in vivo. On the other hand, there is a precedent of participation of Kindlin-2 in transcriptional regulation in tumor-derived cells but also in noncancerous cells, including HK cells.11 In these latter cells Kindlin-2 is recruited by dephosphorylated β-catenin to form a tripartite nuclear complex that transcriptionally activates Wnt target genes.11 Whether Kindlin-2 is also present in complexes that regulate transcription of smad3 target genes remains to be examined.

In summary, the experimental in vitro and in vivo studies by Wei and collaborators describe a novel player in the profibrogenic signaling cascade downstream of TGF-β: The Kindlin-2 protein, which was previously known as a β-integrin adapter that participates in both inside-out and outside-in β-integrin signaling, also functions as a TBR1/smad3 adapter and amplifies TGF-β–driven fibrogenesis in proximal tubular cells and in the kidney.

DISCLOSURES
None.

REFERENCES


See related article, “Kindlin-2 Mediates Activation of TGF-β/Smad Signaling and Renal Fibrosis,” on pages 1387–1398.

Ultrasonic Stimulation of the Cholinergic Anti-Inflammatory Pathway for Renal Protection

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AKI encompasses a large spectrum of pathophysiological processes starting from the early reversible proximal tubular lesion to the definite loss of glomerular filtration and even

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