Searching novel diagnostic markers and targets for therapy of CKD

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Over the last decade, identification and characterization of novel markers of progression and targets for therapy of chronic kidney disease (CKD) have been challenging for the research community. Several promising candidates have emerged, mainly from experimental models of CKD that are yet to be investigated in clinical studies. The authors identified two candidate genes: periostin, an extracellular matrix protein involved in bone and dental development, and the discoidin domain receptor 1 (DDR1), a collagen-binding membrane receptor with tyrosine kinase activity. Both genes are inactive in adulthood under normal conditions but have been shown to be highly inducible following injury to glomerular or tubular epithelial cells. The objective of this review is to summarize recent evidence supporting the role of periostin and DDR1 as potential novel biomarkers and therapeutic targets in CKD.

Key words: DDR1; periostin; renal inflammation and fibrosis

Significant progress has been made in unraveling the molecular mechanisms underlying renal inflammation, fibrosis, and progression of chronic kidney disease (CKD) over the last decade. Yet, no specific treatment has unequivocally been shown to arrest the progression of CKD, which ultimately will require renal replacement therapy in the form of dialysis or kidney transplantation. Several studies have demonstrated that CKD is potentially reversible in animal models by inhibiting the renin–angiotensin system (RAS). However, RAS inhibitors or blockers have not been shown to exhibit the same efficiency in arresting or reversing CKD progression in human studies. Several reasons have been postulated to account for this difference. Animals used in experiments are usually young and healthy, living in pathogen-free environment—factors that cannot be easily controlled for in human studies. The development of kidney disease in animal models is usually rapid and rarely combines multiple mechanisms of injury simultaneously. On the other hand, progression of kidney disease in humans is much more complex and multifactorial. Clearly, the search for novel biomarkers, that will allow for earlier detection of decline in kidney function, and new renoprotective agents beyond RAS blockers is a daunting task, but is desperately needed, given the rising burden of CKD.

Strategy to Identify Diagnostic Markers and Targets for Therapy

The steps involved in identifying potential diagnostic markers and therapeutic targets include the following: (1) establishing a transcriptomic profile of disease progression in a given experimental model or in biopsies of a well-defined cohort of patients; (2) establishing a list of the strongest up- or downregulated genes; (3) extracting from this list the most appealing candidate gene(s); (4) testing the potential involvement of the candidate gene(s) in experimental models by genetic or pharmacological manipulation; and (5) investigating the relevance of the candidate gene(s) in CKD patients. To illustrate this approach, in one of the studies of the authors, a strain of mice overexpressing renin at a constant genetically controlled, high level developed with aging alterations typical of CKD, such as perivascular and periglomerular inflammation, glomerular ischemia, glomerulosclerosis, mesangial expansion, tubular dilation, and loss of foot processes. Transcriptomic analysis during different...
phases of development of renal disease allowed the establishment of a list of several genes with variable expressions depending on the progression (or regression) of disease. Additional selection criteria for the candidate genes were applied: (a) not previously described in the kidney, (b) highly upregulated during progression and reversal to normal levels during therapy, (c) ability to produce proteins that are either circulating or membrane receptors, and (d) similar profile of up- or downregulation in other experimental models of renal disease such as the unilateral ureteral obstruction (UUO) and the nephrotoxic serum-induced models. This strategy led to the identification of two novel kidney proteins that can potentially serve as markers and/or targets for therapy in CKD: periostin and discoidin domain receptor 1 (DDR1).

PERIOSTIN
Periostin (or Osteoblast-Specific Factor 2) is an extracellular protein of 90 kDa, first identified in the periosteum and the periodontal ligament.\(^\text{10}\) It is highly expressed during development and very early in postnatal tissue,\(^\text{11,12}\) however, its expression in healthy adult tissues is low. Angiotensin II can induce periostin expression in fibroblasts and vascular smooth muscle cells via Ras/p38 MAPK/CREB and ERK1/2/TGF-beta1 pathways and via PI3 kinase signaling, respectively.\(^\text{13,14}\) Accordingly, periostin is induced in \textit{in vivo} models mediated by the angiotensin II action such as ischemic, hypertensive, and hypertrophic cardiomyopathies, whereas treatment with an AT1 receptor antagonist decreases the cardiac expression of periostin.\(^\text{15,16}\) Studies in the heart have shown that periostin is secreted by fibroblasts to regulate collagen deposition, thereby altering the mechanical properties of connective tissues.\(^\text{17}\) Periostin has the ability to bind with other extracellular matrix components such as tenasin and fibronectin, and can interact with integrins such as avb3 or avbv, resulting in activation of the Akt or PI3 kinase pathway\(^\text{18}\) (Figure 1a provides an overview of the most common inducers and the target signals of periostin). Interestingly, animals lacking periostin expression exhibit reduced fibrosis after myocardial infarction.\(^\text{19}\) Over the last couple of years, several investigations showed that neoactivation and expression of periostin can occur also in tissues other than the heart. This expression is associated and/or correlated well with inflammation, cell proliferation, or fibrosis and thus can be used as a prognostic/diagnostic marker in various pathological conditions such as breast cancer, asthma, or idiopathic pulmonary fibrosis.\(^\text{20-22}\)

PERIOSTIN AND CKD
In the kidney, studies evaluating the implication of periostin in physiology and disease were scarce until recently. Periostin is transiently expressed during renal development,\(^\text{23}\) and its expression in the normal adult kidney is negligible. In contrast, periostin was shown to be \textit{de novo} expressed in cysts of epithelial cells in human autosomal dominant polycystic kidney.\(^\text{24}\)

![Figure 1](image)

Figure 1 | Physiopathological actions of periostin activation. (a) The ‘periostin network’: \textit{in vitro} data showed that periostin can be highly induced by a variety of signaling pathways; it can interact with integrins to stimulate mechanisms promoting inflammation, extracellular matrix formation, and cell phenotype changes. (b) Proposed mechanism of periostin action in renal epithelial cells following unilateral ureteral obstruction (UUO) injury: periostin is induced early in renal epithelial cells and interacts with the TGF-β signaling pathway to promote inflammation, extracellular matrix remodeling, and subsequently the progression of interstitial fibrosis.
Using models of hypertensive nephropathy induced by L-NNAME or angiotensin II, the authors demonstrated that periostin was highly increased and expressed focally in the extracellular matrix surrounding damaged, inflammatory, and fibrotic renal vessels. Periostin levels declined when the disease was arrested or reversed after treatment with angiotensin II receptor 1 blockers, exhibiting a close correlation with the progression (or regression) of vascular and glomerular lesions. To strengthen this observation, comparison of periostin levels with serum creatinine, proteinuria, and renal blood flow showed that periostin correlated inversely to renal function during progression/regression of CKD. Concomitantly, other investigators showed that periostin levels in biopsies from patients with progressive glomerulopathies such as focal segmental sclerosis or lupus nephritis correlate well with renal damage and decline of estimated glomerular filtration rate. Some studies have demonstrated that periostin is overexpressed in other experimental models of renal pathology (such as diabetic nephropathy or tubulointerstitial fibrosis). More importantly, it has been detected in the urine of patients with CKD, even when albuminuria is low. Subsequent studies investigated the role of periostin as a mediator in the development of renal disease using either mice lacking periostin expression or wild-type mice treated in vivo with antisense oligonucleotides against periostin expression. In both approaches, genetic deletion or inhibition of periostin expression was associated with better preservation of renal structure and function. It is postulated that following kidney injury, early induction of periostin in renal epithelial cells triggers the release of inflammatory chemokines that activate the TGFβ signaling pathway, thereby promoting extracellular matrix remodeling with subsequent progression of interstitial fibrosis.

**PERIOSTIN AS A NOVEL MARKER OF CKD**

An ideal biomarker for CKD should be easily detectable in plasma or urine samples, expressed early in the course of CKD, and should be better or at least comparable to the existing gold standard biomarkers in terms of correlating with the severity of disease. Periostin satisfies several of the above criteria. Current evidence shows that periostin expression is negligible in healthy kidneys but is highly induced in various models of renal disease (UUO, L-NNAME, 5/6 nephrectomy, streptozotocin-induced diabetic nephropathy, nephrotoxic serum nephritis, and renin transgenic mice). In addition, it correlates very well with the degree of tissue damage and the decline of renal function in animal models. Data using transgenic mice expressing the β-galactosidase reporter gene indicate that it is expressed de novo by the cells principally affected in each model with subsequent expression in other cells. In human biopsies, it has been shown to correlate with the decline in GFR. It has been detected in urine samples of CKD patients. Moreover, in experimental models, it has been shown that changes in the levels of periostin occur earlier compared with creatinine or urine albumin. On the basis of the above findings, increased concentration of periostin in the urine may serve as an early marker of renal stress and, inversely, decreased concentration may signal renal function improvement. This hypothesis, however, needs to be investigated in human cohorts.

**PERIOSTIN AS A TARGET FOR THERAPY IN CKD**

Several facets of periostin role in CKD remain to be elucidated, including its mechanism(s) of action, its downstream and upstream mediators, and its paracrine effect on cell population, among others. Available data suggest that, at least in experimental models, genetic deletion of periostin can result in the arrest of several fibrotic or inflammatory pathologies. Furthermore, the observed protection with antisense oligonucleotides provides hope that inhibition of periostin may become a novel target for the treatment of CKD. Several studies are currently investigating pharmacologic agents to test the effect of blocking periostin as potential therapy.

**DISCOIDIN DOMAIN RECEPTOR 1**

DDR1 is a tyrosine kinase transmembrane receptor of collagens, expressed in several cell types and organs, including the gastrointestinal tract, brain, lung, mammary gland, and kidney. The interesting feature of DDR1 is that after the binding of collagens, this receptor is dimerized leading to phosphorylation of tyrosine kinase. Depending on the cellular context, the signaling can be transmitted through the P38 kinase, MAP ER1/2 kinase, PI3 kinase, or JNK pathways, making it the only known collagen receptor with intracellular signaling activity. In vitro studies showed that through the activation of these different pathways, DDR1 can regulate cell differentiation, proliferation, and migration. DDR1 has the potential of being a major mediator of the inflammatory response because in vitro studies showed that it is essential for the maturation and differentiation of monocytes to macrophages. Accordingly, several studies showed that DDR1 is overexpressed in pathological conditions and participates in tissue adaptation to acute and chronic inflammatory lesions by regulating interactions between the extracellular matrix and both resident and infiltrating cells. Its implication in inflammatory processes has already been reported in pulmonary and vascular models of chronic diseases. In addition, a number of studies have shown that overexpression of this receptor is also implicated in cell migration in tumors. DDR1 IN CKD

Recent studies demonstrated that DDR1 is an important mediator in renal inflammation and fibrosis. In a model of angiotensin II-induced hypertensive nephropathy, animals lacking DDR1 expression were protected against proteinuria, perivascular and periglomerular inflammation, glomerulosclerosis, and interstitial fibrosis. Data from studies in the UUO model indicated that DDR1 promotes renal disease through activation of the inflammatory response, as macrophages from DDR1-deleted animals displayed impaired...
stimulate DDR1 and so on. Activation stimulates pro-inflammatory pathways, which in turn
Subsequently, DDR1 is dimerized and phosphorylated, and this cell signal induces locally de novo expression and activation of DDR1. Subsequently, DDR1 is dimerized and phosphorylated, and this activation stimulates pro-inflammatory pathways, which in turn trigger collagen synthesis. Collagens are ligands of DDR1 and further stimulate DDR1 and so on.

DDR1 remains to be elucidated. Once activated, DDR1 expression by administering DDR1 AS A TARGET FOR THERAPY IN CKD

Despite collagen being the most abundant protein in the body, DDR1 is not induced or activated under normal conditions. At present, the events triggering the activation of DDR1 remain to be elucidated. Once activated, DDR1 stimulates inflammatory signaling pathways for collagen synthesis, amplifying the inflammatory and fibrogenic response (Figure 2). Although there is limited understanding of molecular aspects of DDR1, expression and effects of DDR1 remains a promising therapeutic target, as blocking DDR1 activation may limit inflammation and subsequent structural alterations. In has been shown that targeting specifically DDR1 expression by administering in vivo antisense oligonucleotides against DDR1 decreased renal inflammation, blunted proteinuria, and preserved renal function and structure in mice with nephrototoxic serum nephritis. The authors postulated two therapeutic strategies targeting DDR1: (1) inhibiting the binding of collagen to DDR1 through an antibody (Figure 2) and (2) using a specific inhibitor of the tyrosine kinase phosphorylation (Figure 2). Potential limitation for the former approach is the probable difficulty to achieve competitive inhibition against a ubiquitous ligand such as collagen. On the other hand, the latter approach may be limited by the specificity and/or toxicity of tyrosine kinase inhibitors.

CONCLUSION

Accumulating evidence supports the potential role of periostin and DDR1 as promising biomarkers and/or targets of therapy in CKD. Both genes are quiescent in adulthood under physiological conditions but can be induced and activated under pathophysiological conditions. Their activation occurs early after tissue injury. Their de novo expression is restricted to the damaged tissue and appears to trigger or amplify certain phenotypic changes that may lead to the development of CKD. Studies have shown that their inhibition was accompanied by preservation and improvement of renal function in vascular, glomerular, and tubulointerstitial models of renal disease but was not associated with significant detrimental effects in physiologic functions. Despite their potential, the current lack of widely validated tools for application in humans limits their use. The authors remain optimistic that with the growing interest in the field, agents that target periostin and DDR1 will be generated in near future.

DISCLOSURE

All the authors declared no competing interests.

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REFERENCES

1. Lozano R. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012; 380: 2095–2128.
2. Boffa JJ, Ying L, Placier S et al. Regression of renal vascular and glomerular fibrosis: Role of angiotensin II receptor antagonism and metalloproteinases. J Am Soc Nephrol 2003; 14: 1132–1144.
3. Adamczak M, Gross ML, Aman K et al. Reversal of glomerular lesions involves coordinated restructuring of glomerular microvasculature. J Am Soc Nephrol 2004; 15: 3063–3072.
4. Huby AC, Rastaldi MP, Caron K et al. Restoration of podocyte structure and improvement of chronic renal disease in transgenic mice overexpressing renin. PLoS ONE 2009; 21: e6721.
5. Kavvadas P, Weis L, Abed AB et al. Renin inhibition reverses renal disease in transgenic mice by shifting the balance between profibrotic and antifibrotic agents. Hypertension 2013; 61: 901–907.
6. Chatziotanou C, Dussaule IC, Is kidney injury a reversible process? Curr Opin Nephrol Hypertens 2008; 17: 76–81.
7. Maurer M, Zinner B, Gardiner R et al. Renal and retinal effects of enalapril and losartan in type 1 diabetes. N Engl J Med 2009; 361: 40–51.
8. Caron KM, James LR, Kim HS et al. A genetically clamped renin transgene for the induction of hypertension. Proc Natl Acad Sci USA 2002; 99: 8248–8252.
9. Huby AC, Kavvadas P, Alifiari C et al. The RenTg mice: a powerful tool to study hypertension-induced chronic kidney disease. PLoS ONE 2012; 7: e52362.
10. Horiuchi K, Amizuka N, Takeshita S et al. Identification and characterization of a novel protein, periostin, with restricted expression to peristeum and periodontal ligament and increased expression by transforming growth factor beta. J Bone Miner Res 1999; 14: 1239–1249.
11. Kruzyńska-Freitag A, Machnicki M, Rogers R et al. Periostin (an osteoblast-specific factor) is expressed within the embryonic mouse heart during valve formation. Mech Dev 2001; 103: 183–188.
12. Norris RA, Kern CB, Wessels A et al. Identification and detection of the periostin gene in cardiac development. Anat Rec A Discov Mol Cell Biol Evol 2004; 281: 1227–1233.
13. Li L, Fan D, Wang C et al. Angiotensin II increases periostin expression via Ras/p38 MAPK/CREB and ERK1/2/TGF-beta1 pathways in cardiac fibroblasts. Cardiovasc Res 2011; 91: 80–89.
14. Li G, Oparil S, Sanders JM et al. Phosphatidylinositol-3-kinase signaling mediates vascular smooth muscle cell expression of periostin in vivo and in vitro. Atherosclerosis 2006; 188: 292–300.
15. Teekakirikul P, Eminaga S, Toka O et al. Cardiac fibrosis in mice with hypertrophic cardiomyopathy is mediated by non-myocyte proliferation and requires Tgf-beta. J Clin Invest 2010; 120: 3520–3529.
16. Ikekushi K, Taniyama Y, Azuma J et al. Novel mechanisms of valsartan on the treatment of acute myocardial infarction through inhibition of the antiadhesion molecule periostin. Hypertension 2007; 49: 1409–1414.
17. Norris RA, Damon B, Mironov V et al. Periostin regulates collagen fibrillogenesis and the biomechanical properties of connective tissues. J Cell Biochem 2007; 101: 695–711.
18. Butcher JT, Norris RA, Hoffman S et al. Periostin promotes atrioventricular mesenchyme matrix invasion and remodeling mediated by integrin signaling through Rho/PI 3-kinase. Dev Biol 2007; 302: 256–266.
19. Oka T, Xu J, Kaiser RA et al. Genetic manipulation of periostin expression reveals a role in cardiac hypertrophy and ventricular remodeling. Circ Res 2007; 101: 313–321.
20. Xu D, Xu H, Ren Y et al. Cancer stem cell-related gene periostin: a novel prognostic marker for breast cancer. PLoS ONE 2012; 7: e46670.
21. Corren J, Lemanske RF, Hanania NA et al. Lbrzikizumab treatment in adults with asthma. N Engl J Med 2011; 365: 1088–1098.
22. Naik PK, Bozyk PD, Bentley JK et al. COMET Investigators. Periostin promotes fibrosis and predicts progression in patients with idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2012; 303: L1046–L1056.
23. Sorocos K, Kostoulas X, Cullen-McEwen L et al. Expression patterns and roles of periostin during kidney and ureter development. J Urol 2011; 186: 1537–1544.
24. Wallace DP, Quante MT, Reif GA et al. Periostin induces proliferation of human autosomal dominant polycystic kidney cells through alphav-integrin receptor. Am J Physiol Renal Physiol 2008; 295: F1463–F1471.
25. Guerrot D, Dussaule JC, Mael-Ainin M et al. Identification of periostin as a critical marker of progression/reversal of hypertensive nephropathy. PLoS ONE 2012; 7: e31974.
26. Sen K, Lindenmeyer MT, Gaspert A et al. Periostin is induced in glomerular injury and expressed de novo in interstitial renal fibrosis. Am J Pathol 2011; 179: 1756–1767.
27. Satirapoj B, Wang Y, Chamberlin MP et al. Periostin: novel tissue and urinary biomarker of progressive renal injury induces a coordinated mesenchymal phenotype in tubular cells. Nephrol Dial Transplant 2012; 27: 2702–2711.
28. Mael-Ainin M, Abed A, Conway S et al. Inhibition of Periostin expression protects against the development of renal inflammation and fibrosis. J Am Soc Nephrol 2014; 25: 1724–1736.
29. Vogel WF, Abdulhussein R, Ford CE. Sensing extracellular matrix: an update on discoidin domain receptor function. Cell Signal 2006; 18: 1108–1116.
30. Vogel W, Gish GD, Alves F et al. The discoidin domain receptor tyrosine kinases are activated by collagen. Mol Cell 1997; 1: 13–23.
31. Curat CA, Vogel WF. Discoidin domain receptor 1 controls growth and adhesion of mesangial cells. J Am Soc Nephrol 2002; 13: 2648–2656.
32. Hou G, Vogel WF, Bendeck MP. Tyrosine kinase activity of discoidin domain receptor 1 is necessary for smooth muscle cell migration and matrix metalloproteinase expression. Circ Res 2002; 90: 1147–1149.
33. Agarwal G, Milhaj I, Iscrui DF. Interaction of discoidin domain receptor 1 with collagen type 1. J Mol Biol 2007; 367: 443–455.
34. Matsuyama W, Faure M, Yoshimura T. Activation of discoidin domain receptor 1 facilitates the maturation of human monocyte-derived dendritic cells through the TNF receptor associated factor 6/TGF-beta-activated protein kinase 1 binding protein 1 beta/p38 alpha mitogen-activated protein kinase signaling cascade. J Immunol 2003; 171: 3520–3532.
35. Matsuyama W, Kamohara H, Galligan C et al. Interaction of discoidin domain receptor 1 isofrom b (DDR1β) with collagen activates p38 mitogen-activated protein kinase and promotes differentiation of macrophages. FASEB J 2003; 17: 1286–1288.
36. Avivi-Green C, Singal M, Vogel WF. Discoidin domain receptor 1-deficient mice are resistant to bleomycin-induced lung fibrosis. Am J Respir Crit Care Med 2006; 174: 420–427.
37. Franco C, Britto K, Wong E et al. Discoidin domain receptor 1 on bone marrow-derived cells promotes macrophage accumulation during atherosclerosis. Circ Res 2009; 105: 1141–1148.
38. Hidalgo-Carceno C, Hooper S, Chaudhry SI et al. Collective cell migration requires suppression of actomyosin at cell-cell contacts mediated by DDR1 and the cell polarity regulators Par3 and Par6. Nat Cell Biol 2011; 13: 49–58.
39. Park HS, Kim KR, Lee HJ et al. Overexpression of discoidin domain receptor 1 increases the migration and invasion of hepatocellular carcinoma cells in association with matrix metalloproteinase. Oncol Rep 2007; 18: 1435–1441.
40. Flamant M, Placier S, Rodenas A et al. Discoidin domain receptor 1 null mice are protected against hypertension-induced renal disease. J Am Soc Nephrol 2006; 17: 3374–3381.
41. Guerrot D, Kerroch M, Placier S et al. Discoidin domain receptor 1 is a major mediator of inflammation and fibrosis in obstructive nephropathy. Am J Pathol 2011; 179: 83–91.
42. Gross O, Girgent R, Beikowski B et al. Loss of collagen-receptor DDR1 delays renal fibrosis in hereditary type IV collagen disease. Matrix Biol 2010; 29: 346–356.
43. Kerroch M, Guerrot D, Vandermeersch S et al. Genetic inhibition of discoidin domain receptor 1 protects mice against crescentic glomerulonephritis. FASEB J 2012; 26: 4079–4091.