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Research Article

A New Insight into Molecular Function of Smads Signalings in Diabetic Nephropathy

Hiroyuki Ono, Hideharu Abe* and Toshio Doi

Department of Nephrology, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima, Japan

ABSTRACT

Diabetic nephropathy (DN) is the leading cause of end-stage renal failure and is associated with increased morbidity and mortality compared with other causes of renal diseases. Therefore, it is important to elucidate the pathogenesis of DN and establish effective therapies for its treatment. Morphologically, DN is characterized by mesangial matrix expansion caused by the excessive deposition of extracellular matrix proteins such as type IV collagen. Prolonged exposure to hyperglycemia induces advanced glycation end products (AGEs). AGE/RAGE (receptor for AGE) axis induces bone morphogenetic proteins 4 (BMP4) and transforming growth factor-β (TGF-β). Both BMP4/Smad1 and TGF-β/Smad3 signaling pathways are involved in the progression of DN. In particular, Smad1 is the key signaling molecule that is directly involved in the initiation and progression of glomerulosclerosis in DN. BMP4 induces Smad1 and phosphorylation of Smad1 C-terminal domain, its interaction with Smad4, and its translocation into the nucleus, where it regulates the transcription of Col4. However, no study has elucidated the mechanisms underlying the significance of Smad1 linker domain (pSmad1L) in DN. Moreover, the precise role of Smad3 signaling pathway under diabetic conditions is not completely understood, including the correlation between Smad1 and Smad3 signaling. This review article shows that pSmad1L is very important for attenuating DN, and that a new molecular interplay between Smad1 and Smad3 signaling under a diabetic condition might facilitate novel therapeutic agents.

Keywords: Diabetic Nephropathy (DN); Bone Morphogenetic Proteins 4 (BMP4); Smad1; Smad1 linker domain; Smad3; Advanced Glycation End products (AGEs); Transforming growth factor-β (TGF-β)

BACKGROUND

Diabetic nephropathy (DN) is a life-threatening complication of diabetes mellitus and is now the leading cause of end-stage kidney disease worldwide [1]. Proteinuria and progressive renal insufficiency are the characteristic clinical manifestations of DN. Morphologically, DN is characterized by mesangial matrix expansion caused by the excessive deposition of extracellular matrix (ECM) proteins (types I, III, and IV collagens [Col1, Col3, and Col4, respectively]) in the mesangial area [2-5]. During the process of glomerular injuries, mesangial cells (MCs) overproduce Col4 and secrete Col1 and Col3, which are not normally present in the mesangial matrix [6,7]. Excessive synthesis of ECM proteins promotes the development of glomerular sclerosis with renal dysfunction. We previously demonstrated that Smad1 transcriptionally regulates the expression of Col4 in DN [8,9]. Analysis of bone morphogenetic proteins 4 (*Bmp4*) transgenic mice and *Smad1* transgenic mice showed significant induction of glomerular expressions of Smad1, phosphorylation of Smad1 C-terminal domain (pSmad1C), Col4 [10,11]. Thus, Smad1 plays a crucial role of DN progression. Transforming growth factor-β (TGF-β)/Smad3 signaling pathway is also involved in DN [12-14].

ADVANCED GLYCATION END PRODUCTS (AGEs)

Prolonged exposure to hyperglycemia is recognized as the principal cause of diabetic complications [15]. Advanced glycation end products (AGEs) produced as a result of hyperglycemia stimulate the production of ECM proteins [16-18]. In MCs, AGEs induce activation of various signaling pathways. Many previous reports have demonstrated that AGE and its receptor, RAGE, play a critical role in the progression of DN, using various model rodents such as db/db mice [19].

MOLECULAR STRUCTURE OF Smad1

Smad1 is an intracellular molecule that was originally characterized as a signal transducer of the TGF-β superfamily [20]. Smad1 is essential in kidney development [21]. However, the expression of Smad1 is not detected in glomeruli in adult mice [22]. Smad1 consists of two major domains (N-terminal MH1 domain and C-terminal MH2 domain) that are connected by a linker domain. The MH1 domain binds to DNA,whereas the MH2 domain

Correspondence to: Hideharu Abe M.D., Ph.D. Department of Nephrology, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima, 770-8503, Japan, Telephone: +81-88-633-7184, E-mail: abeabe@tokushima-u.ac.jp

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binds to membrane receptors to activate nucleoporins for nuclear translocation and other Smad proteins and nuclear factors to form transcriptional complexes [23].

BMP4/Smad1 SIGNALING

Bone morphogenetic protein 4 (BMP4) is induced by activation of the AGE/RAGE signaling pathway. BMP4 induces Smad1 and the phosphorylation of the Smad1 C-terminal domain (pSmad1C) [10], its interaction with Smad4, and its translocation into the nucleus, where it regulates the transcription of specific target genes [24]. We previously showed that Smad1 transcriptionally regulates the expression of Col4, a major component of excessive mesangial ECM protein deposition in DN, and other ECM proteins such as Col1 and Col3 [8,9]. Actually, *Bmp4* transgenic mice showed significant induction of glomerular expressions of Smad1, pSmad1C, Col4 and Col1 [10]. In addition, TGF-β signaling pathway is activated downstream of the AGE/RAGE signaling pathway as previously reported by other groups and our own data [12-14].

Members of the TGF-β superfamily bind to two different types of serine/threonine kinase receptors, termed type I and type II receptors [25]. Type II receptors activate type I receptors, which transduce various signals via the Smads. Two type I receptors have been described for TGF-β, activin receptor-like kinase type 1 (ALK1) and type 5 (ALK5) [26]. We previously showed that the expression of ALK1 was induced in AGE-treated MCs. Moreover, ALK1, together with Smad1 and Col4, was highly expressed in human DN, corresponding to the progression of diabetic conditions [8].

However, the precise role of the Smad3 signaling pathway, which is activated downstream of the TGF-β signaling pathway under diabetic condition, is not completely understood.

Thus, Smad1 is the key signaling molecule directly involved in the initiation and progression of glomerulosclerosis in DN and other kidney diseases [10,27]. Some research has demonstrated that the phosphorylation of Smad1 linker domain (pSmad1L) prevents the nuclear translocation of Smad1, thus inactivating Smad1 signaling in Xenopus embryogenesis and mouse stem cells [28]. Moreover, the correlation between Smad1 and Smad3 signaling is unclear. We recently examined our working hypothesis that phosphorylation of the Smad1 linker domain leads to inactivation of Smad1 and subsequently suppresses progression of DN.

NEW FINDINGS OF pSmad1L FUNCTION AND INTERACTION BETWEEN Smad1 AND Smad3 SIGNALING

We conducted a long-term *in vivo* study using genetically diabetic (db/db) mice and *Smad3*-knockout diabetic mice. We made *Smad3*$^{+/-}$;db/db mice and compared them with db/db mice. *Smad3*$^{+/-}$;db/db mice showed partial improvement of albuminuria and significant decrease in expression of ECM proteins such as Col4, Col1, and Col3 compared with db/db mice. The levels of pSmad1C and pSmad1L decreased and increased, respectively, in *Smad3*$^{+/-}$;db/db mice compared with the levels observed in db/db mice. Phosphorylation of the Smad3 C-terminal and linker domains was attenuated in *Smad3*$^{+/-}$;db/db mice, compared with that in db/db mice [29]. These data suggest that altered Smad1 phosphorylation in relation to Smad3 expression in the diabetic glomeruli plays important roles in DN progression.

We prepared constructs of mutant of Smad1 (Smad1 (S206E) as a constitutively active form and Smad1 (S206A) as a dominant negative form) (Figure 1), and these constructs were transfected into MCs. AGE stimulation was used in MCs to induce a diabetic condition. pSmad1C levels significantly decreased, and Smad3, pSmad3C, and pSmad3L levels remained unchanged in AGE-treated MCs overexpressing pSmad1L, indicating that Smad1 linker domain activation suppressed Smad1 activation without affecting Smad3 expression and activation under diabetic conditions [29]. In contrast, pSmad1C levels significantly decreased and pSmad1L levels significantly increased in AGE-treated *Smad3*-null MCs [29]. Together, these results suggest that Smad3 expression and activation exert an important effect on Smad1 activation and subsequent ECM protein overexpression in DN. Moreover, pSmad3C overexpression significantly increased pSmad1C level and decreased pSmad1L level without affecting Smad1 and pSmad3L expression in AGE-treated MCs [29].

URINARY Smad1 AS A BIOMARKER FOR EARLY PHASE OF DN

The most reliable diagnostic procedure is renal biopsy, but it is impossible to perform biopsies for all patients with DN. Increased urinary protein excretion may be an early clinical manifestation of diabetic nephropathy [30]. To date, the measurement of albuminuria has been used as a standardized non-invasive test for the diagnosis of early DN [31]. However, in some cases, albuminuria does not correlate with glomerulosclerosis at all in the early phase of DN. Therefore, it is important to establish practical approaches to the diagnosis by novel diagnostic markers specific for the detection of mesangial expansion in the early phase of DN. There was a very good correlation between urinary Smad1 levels and the development of mesangial expansion in diabetic rats [32].

SUMMARY

Smad1 is the key signaling molecule directly involved in progression of glomerulosclerosis in DN. Earlier diagnosis may lead to better long-term outcomes for patients with DN. Recent clinical studies demonstrated that urinary Smad1 could be an early predictor in diabetic patients [33]. Thus, Smad1 could be useful for diagnosis of DN as well as understanding of pathophysiology in DN.

We have recently revealed that Smad1 signaling is controlled by Smad3 expression, and that phosphorylation of the Smad1 linker domain leads to inactivation of Smad1 and subsequently suppresses progression of DN [29]. Knocking out Smad3 upregulates phosphorylation of the Smad1 linker domain, while downregulating phosphorylation of the C-terminal domain, and this leads to prevent Smad1 from going to the nucleus to impact on gene expression, especially those involved in glomerulosclerosis. Thus, Smad3 is potential therapeutic targets for DN, but the direct inhibition of Smad3 causes undesirable adverse effects such as cancer [34,35]. On the other hand, preferential activation of the Smad1 linker domain may provide a new therapeutic approach for treatment of DN.

In conclusion, we clarified a new interaction between Smad1 and Smad3 signaling under diabetic conditions and found that phosphorylation of the Smad1 linker domain may play a crucial role in DN progression (Figure 2).

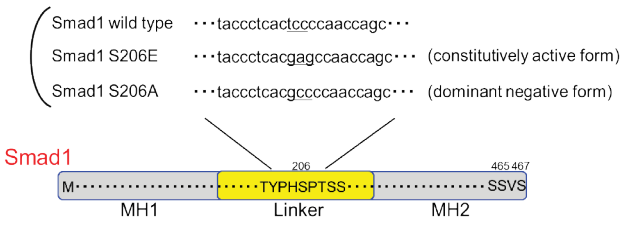

Figure 1: Mammalian expression constructs of mutant versions of Smad1.

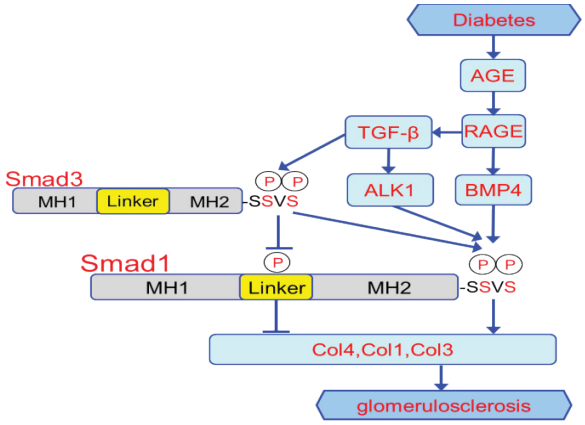

Figure 2: Proposal molecular model of diabetic nephropathy.

REFERENCES

1. Molitch ME, DeFronzo RA, Franz MJ, Keane WF, Mogensen CE, Parving HH, et al. (2004) Nephropathy in diabetes. Diabetes Care. 2004;27:S79-83.
2. Mason RM, Wahab NA Extracellular matrix metabolism in diabetic nephropathy. J Am Soc Nephrol. 2003;14:1358-73.
3. Striker LJ, Doi T, Elliot S, Striker GE. The contribution of glomerular mesangial cells to progressive glomerulosclerosis. Semin Nephrol. 1989;9:318-28.
4. Striker LJ, Peten EP, Elliot SJ, Doi T, Striker GE. Mesangial cell turnover: effect of heparin and peptide growth factors. Lab Invest. 1991;64:446-56.
5. Stokes MB, Hudkins KL, Zaharia V, Taneda S, Alpers CE. Up-regulation of extracellular matrix proteoglycans and collagen type I in human crescentic glomerulonephritis. Kidney Int. 2001;59:532-42.
6. Bruggeman LA, Burbelo PD, Yamada Y, Klotman PE. A novel sequence in the type IV collagen promoter binds nuclear proteins from Engelbreth-Holm-Swarm tumor. Oncogene. 1992;7: 1497-502.
7. Ziyadeh FN, Hoffman BB, Han DC, Iglesias-De La Cruz MC, Hong SW, Isono M, et al. Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-beta antibody in db/db diabetic mice. Proc Natl Acad Sci U S A. 2000;97:8015-20.
8. Abe H, Matsubara T, Iehara N, Nagai K, Takahashi T, Arai H, et al. Type IV Collagen Is Transcriptionally Regulated by Smad1 under Advanced Glycation End Product (AGE) Stimulation. J Biol Chem. 2004;279: 14201-206.
9. Matsubara T, Abe H, Arai H, Nagai K, Mima A, Kanamori H, et al. Expression of Smad1 is directly associated with mesangial matrix expansion in rat diabetic nephropathy. Lab Invest. 2006;86:357-68.
10. Tominaga T, Abe H, Ueda O, Goto C, Nakahara K, Murakami T, et al. Activation of bone morphogenetic protein 4 signaling leads to glomerulosclerosis that mimics diabetic nephropathy. J Biol Chem. 2011;286:20109-116.
11. Matsubara T, Araki M, Abe H, Ueda O, Jishage K, Mima A, et al. Bone Morphogenetic Protein 4 and Smad1 Mediate Extracellular Matrix Production in the Development of Diabetic Nephropathy. Diabetes. 2015;64:2978-90.
12. Flyvbjerg A, Denner L, Schrijvers BF, Tilton RG, Mogensen TH, Paludan SR, et al. Long-term renal effects of a neutralizing RAGE antibody in obese type 2 diabetic mice. Diabetes. 2004;53:166-72.
13. Lan KC, Chiu CY, Kao CW, Huang KH, Wang CC, Huang KT, et al. Advanced glycation end-products induce apoptosis in pancreatic islet endothelial cells via NF-?B-activated cyclooxygenase-2/prostaglandin E2 up-regulation. PLoS One . 2015;10:e0124418.
14. Abe H, Tominaga T, Matsubara T, Abe N, Kishi S, Nagai K, et al. Scleraxis modulates Bone Morphogenetic Protein 4 (BMP4)-Smad1 protein-Smooth Muscle a-Actin (SMA) signal transduction in diabetic nephropathy. J Biol Chem. 2012;287:20430-42.
15. Pirart J Diabetes Mellitus and Its Degenerative Complications: A Prospective Study of 4,400 Patients Observed between 1947 and 1973 (Pt 1). Diabetes Care. 1978;1:168-88.
16. Doi T, Vlassara H, Kirstein M, Yamada Y, Striker GE, Striker LJ. Receptor-specific increase in extracellular matrix production in mouse mesangial cells by advanced glycosylation end products is mediated via platelet-derived growth factor. Proc Natl Acad Sci U S A. 1992;89: 2873-7.
17. Iehara N, Takeoka H, Yamada Y, Kita T, Doi T. Advanced glycation end products modulate transcriptional regulation in mesangial cells. Kidney Int. 1996;50:1166-72.
18. Bohlender JM, Franke S, Stein G, Wolf G. Advanced glycation end products and the kidney. Am J Physiol Renal Physiol. 2005;289:F645-59.
19. Forbes JM, Cooper ME, Oldfield MD, Thomas MC. Role of advanced glycation end products in diabetic nephropathy. J Am Soc Nephrol. 2003;14:S254-8.
20. Liu F, Hata A, Baker JC, Doody J, Cárcamo J, et al. A human Mad protein acting as a BMP- regulated transcriptional activator. Nature. 1996;381:620-3.
21. Roelen BA, van Rooijen MA, Mummery CL. Expression of ALK-1, a type 1 serine/threonine kinase receptor, coincides with sites of vasculogenesis and angiogenesis in early mouse development. Dev Dyn. 1997;209:418-30.
22. Huang S, Flanders KC, Roberts AB. Characterization of the mouse Smad1 gene and its expression pattern in adult mouse tissues. Gene. 2000;258:43-53.
23. Heldin CH, Miyazono K, ten Dijke P. TGF-beta signalling from cell membrane to nucleus through SMAD proteins. Nature. 1997;390:465-71.
24. Shi Y, Massagué J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell. 2003;113:685-700.
25. Sugiyama S, Miyata T, Horie K, Iida Y, Tsuyuki M, Tanaka H, et al. Advanced glycation end-products in diabetic nephropathy. Nephrol Dial Transplant. 1996;11:91-4.
26. ten Dijke P, Miyazono K, Heldin CH. Signaling via hetero-oligomeric complexes of type I and type II serine/threonine kinase receptors. Curr Opin Cell Biol. 1996;8:139-45.
27. Araki M, Matsubara T, Abe H, Torikoshi K, Mima A, Lehara N, et al. Conditional deletion of Smad1 ameliorates glomerular injury in progressive glomerulonephritis. Sci Rep. 2016;6:31216.
28. Sapkota G, Alarcón C, Spagnoli FM, Brivanlou AH, Massagué J. Balancing BMP signaling through integrated inputs into the Smad1 linker. Mol Cell. 2007;25:441-54.
29. Ono H, Abe H, Sakurai A, Ochi A, Tominaga T, Tamaki M, et al. Novel Interplay Between Smad1 and Smad3 Phosphorylation via AGE Regulates the Progression of Diabetic Nephropathy. Sci Rep. 2018;12:10548.
30. Mogensen CE. Prediction of clinical diabetic nephropathy in IDDM patients. Alternatives to microalbuminuria? Diabetes. 1990;39:761-7.
31. Caramori ML, Fioretto P, Mauer M. The need for early predictors of diabetic nephropathy risk: is albumin excretion rate sufficient? Diabetes. 2000;249:1399-408.

32. Mima A, Arai H, Matsubara T, Abe H, Nagai K, Tamura Y, et al. Urinary Smad1 is a novel marker to predict later onset of mesangial matrix expansion in diabetic nephropathy. Diabetes. 2008;57:1712-22.

33. Doi T, Moriya T, Fujita Y, Minagawa N, Usami M, Sasaki T, et al. Urinary IgG4 and Smad1 Are Specific Biomarkers for Renal Structural and Functional Changes in Early Stages of Diabetic Nephropathy. Diabetes. 2018;67:986-93.

34. Wolfraim LA, Fernandez TM, Mamura M, Fuller WL, Kumar R, Cole DE, et al. Loss of Smad3 in acute T-cell lymphoblastic leukemia. N Engl J Med. 2004;351:552-9.

35. Ku JL, Park SH, Yoon KA, Shin YK, Kim KH, Choi JS, et al. Genetic alterations of the TGF-beta signaling pathway in colorectal cancer cell lines: A novel mutation in Smad3 associated with the inactivation of TGF-beta-induced transcriptional activation. Cancer Lett. 2007;247:283-92.