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

Hiroyuki Ono, Hideharu Abe* and Tobio 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)

Introduction

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 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].

*Corresponding author: Hideharu Abe, MD, Ph D. Department of Nephrology, Institute of Health Biosciences, University of Tokushima Graduate School, Tokushima, 770-8503, Japan, Tel: +81-88-633-7184; E-mail: abeabe@tokushima-u.ac.jp

Received November 29, 2018; Accepted January 02, 2019; Published January 09, 2019


Copyright: © 2019 Ono H, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
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 I (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


