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Histone deacetylase 4 selectively contributes to podocyte injury in diabetic nephropathy

Xiaojie Wang[1], Jiang Liu[1], Junhui Zhen[2], Chun Zhang[3], Qiang Wan[4], Guangyi Liu[5], Xinbing Wei[1], Yan Zhang[1], Ziying Wang[1], Huirong Han[1], Huiyan Xu[1], Chanchan Bao[6], Zhenyu Song[1], Xiumei Zhang[1], Ningjun Li[7] and Fan Yi[1]

[1]Department of Pharmacology, Shandong University School of Medicine, Jinan, China; [2]Department of Pathology, Shandong University School of Medicine, Jinan, China; [3]Department of Nephrology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; [4]Department of Nephrology, Provincial Hospital Affiliated to Shandong University, Jinan, China; [5]Department of Nephrology, Qilu Hospital, Shandong University, Jinan, China; [6]The Microscopy Characterization Platform, Shandong University, Jinan, China and [7]Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia, USA

Studies have highlighted the importance of histone deacetylase (HDAC)-mediated epigenetic processes in the development of diabetic complications. Inhibitors of HDAC are a novel class of therapeutic agents in diabetic nephropathy, but currently available inhibitors are mostly nonselective inhibit multiple HDACs, and different HDACs serve very distinct functions. Therefore, it is essential to determine the role of individual HDACs in diabetic nephropathy and develop HDAC inhibitors with improved specificity. First, we identified the expression patterns of HDACs and found that, among zinc-dependent HDACs, HDAC2/4/5 were upregulated in the kidney from streptozotocin-induced diabetic rats, diabetic db/db mice, and in kidney biopsies from diabetic patients. Podocytes treated with high glucose, advanced glycation end products, or transforming growth factor-β (common detrimental factors in diabetic nephropathy) selectively increased HDAC4 expression. The role of HDAC4 was evaluated by *in vivo* gene silencing by intrarenal lentiviral gene delivery and found to reduce renal injury in diabetic rats. Podocyte injury was associated with suppressing autophagy and exacerbating inflammation by HDAC4-STAT1 signaling *in vitro*. Thus, HDAC4 contributes to podocyte injury and is one of critical components of a signal transduction pathway that links renal injury to autophagy in diabetic nephropathy.

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Correspondence: *Fan Yi, Department of Pharmacology, Shandong University School of Medicine, 44#, Wenhua Xi Road, Jinan, Shandong, 250012, China. E-mail: fanyi@sdu.edu.cn*

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Although the pathogenesis of diabetic nephropathy (DN) is multifactorial, an increasing number of experimental studies have highlighted the importance of histone deacetylase (HDAC)-mediated epigenetic processes in the development of renal injury.[1–4] HDACs are a family of enzymes that balance the acetylation activities of histone acetyltransferases on chromatin remodeling and have essential roles in regulating gene transcription.[5] Currently, 18 HDAC genes are identified and classified into four groups depending on sequence identity and domain organization. Class I (HDACs 1, 2, 3, and 8), class II (HDACs 4, 5, 6, 7, 9, and 10), and class IV (HDAC 11) are Zn^{2+}-dependent for enzymatic activity, whereas the class III sirtuins (SIRT1–7) act through a distinct NAD^{+}-dependent mechanism. HDAC inhibitors have been reported to have anti-inflammatory and antifibrotic effects in the kidney. Administration of the HDAC inhibitor valproic acid has beneficial effects on proteinuria, glomerulosclerosis, and renal inflammation in the experimental mouse adriamycin-induced nephropathy model.[1] Inhibition of HDAC activity suppresses epithelial-to-mesenchymal transition induced by transforming growth factor-β (TGF-β) in human renal epithelial cells[6] and in streptozotocin (STZ)-induced diabetic kidneys.[7] A recent study shows that long-term treatment with the HDAC inhibitor vorinostat improves albuminuria and mesangial matrix accumulation in diabetic mice through an endothelial nitric oxide synthase-dependent mechanism.[8] Although these findings suggest that HDACs contribute to the pathogenesis of renal disease and provide further support to a growing body of evidence attesting to the potential utility of HDAC inhibitors for the treatment of renal diseases, currently available HDAC inhibitors are mostly nonselective (pan-inhibitors) and inhibit multiple HDAC proteins, and different HDACs serve very distinct functions. In addition, prolonged broad-spectrum HDAC inhibition using pan-HDAC inhibitors might involve risk not only because these substances have been associated with adverse side effects but also because relatively little is known regarding the function

of individual HDACs. Therefore, it is important to elucidate the functional role of individual HDACs in DN and develop HDAC inhibitors with improved specificity. By targeting only the most relevant HDAC isoform in a particular indication, it may be possible to greatly improve the efficacy by removing certain toxicities that may be associated with the inhibition of multiple isoforms.

Autophagy is emerging as an important pathway in many biological processes and diseases.[9] Although studies have shown that autophagy regulates many critical aspects of normal and disease conditions in the kidney, such as tubular injuries, kidney development and aging, genetic diseases associated with the kidney, and DN,[10–12] the importance of autophagy in the kidney has only just begun to be elucidated. Autophagy has an essential role in stress adaption in renal injuries through removal of protein aggregates and damaged organelles, and promotion of cell survival; autophagy can also contribute to cell death by autophagic cell death. However, how the process of autophagy is altered in the pathogenesis of renal diseases and how this alteration is beneficial or detrimental to renal functions is unclear.[9] It is being increasingly recognized that acetylation can also regulate autophagy.[13] Increased cellular acetylation level by HDAC inhibition in cells promotes autophagy,[14] and knockdown of the histone acetyltransferase KAT2B/p300 induces autophagy in nutrient-rich conditions.[15,16] In this study, we identified for the first time the expression patterns of HDACs in DN and found that HDAC4 selectively contributes to podocyte injury by exacerbating inflammation and suppressing autophagy.

RESULTS

Expression patterns of HDACs in kidneys of STZ-induced diabetic rats, in kidneys of diabetic db/db mice, and in renal biopsies from DN patients

As shown in Table 1, STZ-induced diabetic rats had hyperglycemia and lower body weight compared with their nondiabetic counterparts. No difference in blood pressure was observed between these groups. Urinary albumin excretion was significantly increased in diabetic rats. Real-time RT-PCR (Figure 1a) and western blot (Figure 1b) analyses showed that, among Zn^{2+}-dependent HDACs, the expression levels of HDAC2, HDAC4, and HDAC5 were significantly increased in the kidneys of STZ-induced diabetic rats. The levels of HDAC1, 3, 6, 7, 8, 9, 10, and 11 had no significant changes. Our immunohistochemical studies further confirmed the upregulation of HDAC2, HDAC4, and HDAC5 in the kidneys of STZ-induced diabetic rats. Under high magnification it was shown that HDAC2 was enriched in the renal tubular and interstitial area; HDAC4 was upregulated in glomerular capillaries, whereas HDAC5 was mainly present in the mesangial area in DN animals (Figure 1c). We also observed the enhanced renal expressions of HDAC2, HDAC4, and HDAC5 in another *in vivo* model of diabetes, the db/db mice, indicating that HDAC2, HDAC4, and HDAC5 may be the common pathogenic factors in both type 1 and type 2 *in vivo* animal models of diabetes (Figure 1d).

Consistent with the changes seen in animal studies, upregulation of HDAC2, HDAC4, and HDAC5 was also observed in paraffin-embedded sections of human diabetic renal tissues on immunohistochemical staining, but weak expression was seen in the kidneys from normal controls or diabetic patients without nephropathy (Figure 2a). Real-time RT-PCR analysis further confirmed the changes in mRNA levels of HDAC2 (Figure 2b), HDAC4 (Figure 2d), and HDAC5 (Figure 2f) in renal biopsies from DN subjects. HDAC2 expression level showed an increasing tendency, although there was no statistical difference. In addition, in these available samples, we found that HDAC2 (Spearman's $r = -0.4044$, $P < 0.05$; Figure 2c), HDAC4 (Spearman's $r = -0.7740$, $P < 0.01$; Figure 2e), and HDAC5 (Spearman's $r = -0.4120$, $P < 0.05$; Figure 2g) mRNA levels were negatively correlated with the estimated glomerular filtration

Table 1 | Physical and biochemical parameters of the experimental animals

	Control		STZ-induced diabetic rats	
Variable	pGLV3-null	pGLV3-shRNA2-HDAC4	pGLV3-null	pGLV3-shRNA2-HDAC4
Body weight (g)				
0 Weeks	221.6 ± 18.3	216.9 ± 11.6	231.1 ± 13.2	228.3 ± 15.7
12 Weeks	424.2 ± 31.9	419.6 ± 26. 7	275.2 ± 31.6[a]	299.8 ± 34.9[a]
Heart rate (beat/min)	443.9 ± 16.2	457.1 ± 20. 8	423.1 ± 28.7	434.7 ± 19. 6
Blood pressure (mm Hg)				
Systolic	108.2 ± 6.2	107.1 ± 10.3	104.5 ± 9.8	105.7 ± 11.4
Diastolic	67.7 ± 8.2	70.2 ± 9.3	68.5 ± 7.5	66.4 ± 10.6
Glucose (mmol/l)	5.3 ± 0.6	6.1 ± 0.7	26.4 ± 4. 8[a]	24.4 ± 5.5[a]
Urine volume (ml/24 h)	18.2 ± 5.3	21.07 ± 6.4	168.9 ± 22.1[a]	101.8 ± 31.2[a,b]
Urinary albumin (mg/24 h)	4.3 ± 0.6	5.1 ± 0.7	27.4 ± 6.9[a]	13.9 ± 5.3[a,b]

Abbreviations: HDAC, histone deacetylase; STZ, streptozotocin.
Male Sprague–Dawley rats were uninephrectomized. After a 1-week recovery period from uninephrectomy, diabetes was induced by tail-vein injection of streptozotocin (STZ) at 50 mg/kg body wt in sodium citrate buffer. A day after STZ injection, recombinant lentivirus vectors harboring a short-hairpin RNA sequence targeting HDAC4 (pGLV3-shRNA-HDAC4) or their negative controls (pGLV3-null) were delivered into the rat kidney by means of intraparenchymal injections. All rats had unrestricted access to food/water and were maintained for 12 weeks.
Values are means ± s.e.m. for 10 mice in each group.
[a]$P < 0.05$ versus normal rats.
[b]$P < 0.05$ versus STZ-induced diabetic rats.