TGF-β induces miR-30d down-regulation and podocyte injury through Smad2/3 and HDAC3-associated transcriptional repression

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Abstract
The microRNA-30 family plays important roles in maintaining kidney homeostasis. Patients with focal segmental glomerulosclerosis (FSGS) have reduced miR-30 levels in glomerulus. TGF-β represses miR-30s in kidney podocytes, which leads to cytoskeleton damage and podocyte apoptosis. In this study, we investigated the mechanism by which TGF-β represses miR-30d in vitro. The human miR-30d promoter contains multiple copies of Smad binding element-like sequences. A fragment of 150 base pairs close to the transcription start site was negatively regulated by TGF-β to a similar extent as the 1.8 kb promoter, which was blocked by histone-deacetylase inhibition. TGF-β specifically enhanced HDAC3 expression. Knockdown of HDAC3 by shRNA or a selective inhibitor RGFP966 significantly relieved the repression of miR-30d mRNA and the promoter transcription. TGF-β promoted HDAC3 association with Smad2/3 and NCoR and caused their accumulation at the putative Smad binding site on the miR-30d promoter, which was prohibited by TSA or RGFP966. Furthermore, TSA or RGFP966 treatment reversed TGF-β-induced up-regulation of miR-30d targets Notch1 and p53 and alleviated the podocyte cytoskeleton damage and apoptosis. Taken together, these findings pinpoint that TGF-β represses miR-30d through a Smad2/3-HDAC3-NCoR repression complex and provide novel insights into a potential target for the treatment of podocyte injury-associated glomerulopathies.

Key message
- MiR-30d promoter is negatively regulated by TGF-β.
- TGF-β down-regulates miR-30 through Smad signaling pathway.
- HDAC3 and NCoR are recruited by Smad2/3 to mediate miR-30d repression by TGF-β.
- HDAC3 acts as a critical player in TGF-β-induced miR-30d repression and podocyte injuries.

Keywords TGF-β · miR-30d · HDAC3 · Smad · Podocyte · Transcriptional repression

Introduction
MicroRNAs (miRNAs) are a class of small endogenous non-coding RNAs that act as posttranscriptional regulators of gene expression in animals and plants [1]. Generally, miRNAs mediate translational repression or messenger RNA degradation by targeting the 3'-untranslated region (3'-UTR) of messenger RNAs through pairing interaction. The miR-30 family consists of five members (miR-30a, b, c, d, and e) that regulate many cellular processes, including cell growth suppression [2], epithelial-to-mesenchymal transition (EMT) [3], and adipogenic differentiation [4]. Recent studies have revealed that miR-30 is abundant in kidney podocyte. Patients with
podocyte damage such as focal segmental glomerulosclerosis (FSGS) have reduced miR-30s in glomerulus. In vitro sustained miR-30 expression protects podocytes from TGF-β-induced injuries, while knockdown of miR-30 causes podocyte cytoskeleton damage and apoptosis [5]. The miR-30 family members are encoded from three different genomic locations and form three miRNA clusters, in which miR-30b and miR-30d are encoded by a single transcript [6]. Although miR-30 members display the similar expression and regulatory patterns, its regulation mechanism is still largely unknown, partially due to the uncertainty of their promoter locations.

It’s generally accepted that TGF-β is a key regulator of kidney pathogenesis. TGF-β induces many cellular responses through either Smad-dependent or Smad-independent signaling pathways. Interestingly, Smad signaling can both transactivate and repress the target gene expression in the same cell, depending on what transcription accessory proteins are recruited [7, 8]. In addition to the protein-encoding genes, TGF-β also regulates the expression of numerous miRNAs [9, 10].

Acetylation and deacetylation of nucleosomal histone proteins are important epigenetic modifications that regulate transcriptional activity. Histone acetyl-transferase (HAT) and histone deacetylase (HDAC) reversely modify the acetylation state of lysine residues in nucleosomal histones, thereby controlling the chromatin conformation and affecting the gene transcription [11, 12]. Namely, HATs promote an open chromatin configuration and transcriptional activation, while HDACs generally promote chromatin condensation and transcriptional repression. HDACs are well known for recruiting transcription repressors such as SMRT or NCoR and negatively regulating target gene transcription [13]. A growing body of evidence reveals that expressions of miRNAs are also subjected to histone acetylation regulation [14, 15]. The expression of several miRNAs such as miR-200b [16], miR-183 [17], and miR-15a/16-1 [18] is influenced by HDAC-mediated transcriptional repression, although the underlying molecular mechanisms are not fully explored.

MiR-30d promoter has been recently identified [19]; however, very little is known about its transcriptional regulation. In this study, we seek to explore the mechanism by which TGF-β represses miR-30d. Our study indicates that TGF-β represses miR-30d through Smad signaling pathway. Smad 2/3 form a transcription repression complex containing HDAC3 and NCoR that acts on miR-30d promoter and suppresses miR-30d transcription. The results also suggest that HDAC3 is a critical player in Smad2/3-orchestrated miR-30 down-regulation and subsequent podocyte damage.

Materials and methods

Antibodies and reagents

HDAC Antibody Sampler Kit (9928 s), p53, Notch1, horse anti-mouse IgG, and goat anti-rabbit IgG antibodies were from Cell Signaling Technology (USA); Anti-NCoR, anti-acetylated histone3 (Millipore, USA), anti-Smad2/3 antibody (Santa Cruz, Biotech, USA), Recombinant human TGF-β1, trichostatin A (TSA) (Sigma Aldrich, USA), and RGFP966 (Selleck) were from indicated providers. The 3TP-luc plasmid was a gift from Dr. Joan Massaque (Memorial Sloan Kettering Cancer Center, USA).

Cell cultures and transfection

Human podocytes [20] and human embryo kidney cells HEK293 (ATCC, USA) were maintained in Dulbecco’s modified Eagle’s medium with 10 % fetal bovine serum and 1 % penicillin/streptomycin (GIBCO) at 37 °C in a humidified atmosphere of 5 % CO₂. Lipofectamine 2000 (Invitrogen, USA) was used for transient transfection following the manufacturer’s instructions.

Quantitative real-time PCR of microRNA

Total RNA was extracted with TRIzol reagent (Invitrogen, USA). The reverse transcription and quantitative real-time polymerase chain reaction (qRT-PCR) were performed with a qRT-PCR kit (Takara Bio, Japan). The primers specific for miR-30s were synthesized from Invitrogen. U6 RNA was used for normalization. The PCR Primers are listed in Table 1.

Plasmid construction

MiR-30d promoter was identified recently [19]. Initially, two fragments of miR-30d promoter (30dP1, from −1799 to +229, and 30dP4, from −338 to +229) were cloned from genomic DNA of HEK293 cells. The PCR fragments were inserted into pGL3 enhancer vector (Promega, USA) at the MluI and XhoI sites. The 30dP1 fragment contains an internal EcoRI (–1078) and a PvuII site (–1006), respectively. The promoter truncation mutants 30dP2 and 30dP3 were generated by excising MluI/EcoRI or MluI/PvuII/fragment, respectively, from 30dP1. The 30dP2* and 30dP3* were generated by excising EcoRI/XhoI or PvuII/XhoI fragments from 30dP1 as illustrated in Fig. 2a. A flag-tagged HDAC3 expression plasmid was a generous gift from Dr. Alan Friedman (Johns Hopkins Oncology Center, USA).
Luciferase reporter assay

Cells were transfected with reporter plasmids and a renilla luciferase plasmid (pRL-SV40, Promega, USA) as internal control. At 8 h posttransfection, TGF-β (5 ng/ml), RGFP966 (10 μM), or TSA (30 ng/ml) was added to cells. After 24 h, the luciferase activity was assessed from harvested total cell lysates using a dual luciferase assay system (Promega, USA).

Chromatin immunoprecipitation (ChIP)

Chromatin-immune-precipitation (ChIP) assay was performed using the EZ ChIP Chromatin Immuno-precipitation Kit (Millipore, USA) according to the manufacturer’s instructions. The immune-precipitations were performed with 2 μg antibodies specific for Smad2/3, HDAC3, NCoR, Acetyl-Histone H3, or an IgG isofom control at 4 °C overnight with rotation. After de-crosslinking and DNA extraction, the antibody-associated DNA fragments were subjected to PCR amplification with primer sets that covered the tentative Smad binding elements on miR-30d promoter (ChIP-pos F/R, −344 to +14) or negative control (ChIP-neg F/R, −1799 to −1498. The primer sequences were provided in Table 1). PCR products were analyzed by 1 % agarose electrophoresis and visualized under UC light.

Western blot

Western blot was performed as previously described [5, 21].

Co-immunoprecipitation

Co-immunoprecipitation experiments were performed essentially as before [21] with antibodies specific for Smad2/3, NCoR, Flag, or isoform matched IgGs. After precipitation, the immunoprecipitants were analyzed by immunoblotting using antibodies against Flag, Smad2/3, or NCoR, respectively.

RNA interference

HDAC3 knockdown was performed with an HDAC3-shRNA plasmid constructed in GV102 vector. The oligo sequences were illustrated in Table 1. A plasmid containing a scrambled sequence was used as control.

F-actin cytoskeleton staining

Human podocytes [5] were treated with TGF-β with or without TSA/RGFP966 for 24 h. Cells were fixed with 4 % paraformaldehyde for 20 min at room temperature. After washes, cells were permeabilized with 0.1 % Triton X-100 for 5 min, then blocked with 1 % BSA. F-actin was stained with rhodamine-labeled phalloidin. Fluorescence images were visualized with a fluorescence microscope. Quantification of rhodamine stained F-actin fibers was performed with Image J software.

Annexin V flow cytometry analysis of apoptosis

Human podocytes were treated with TGF-β, TSA, or RGFP966 for 24 h. After washes with ice-cold PBS, the cells were resuspended in Annexin V binding buffer and incubated with FITC-conjugated Annexin V antibody and propidium iodide (1:100 dilutions) for 15 min at room temperature. The cells were then analyzed with a Beckman Counter (MoFlo XDP).

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**Table 1** Primers

<table>
<thead>
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<th>Primer list</th>
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<td>miR-30d</td>
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<tr>
<td>U6-forward primer</td>
<td>CTCGCCATCCGAGCA</td>
</tr>
<tr>
<td>U6-reverse primer</td>
<td>AAGCTTCAATGTTGCTGG</td>
</tr>
<tr>
<td>P1-forward primer</td>
<td>CCGCTCGAGAGATGGAATG</td>
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<td>P1-reverse primer</td>
<td>CCAAGCTTGAGTCATAG</td>
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<td>P4-forward primer</td>
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<td>ChIP-neg-forward primer</td>
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<tr>
<td>ChIP-neg-reverse primer</td>
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<td>ChIP-pos-forward primer</td>
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<tr>
<td>ChIP-pos-reverse primer</td>
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<tr>
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Statistics

Data are presented as the mean±standard deviation (S.D.). The difference between the two groups was analyzed with Student’s t test. \( p<0.05 \) was considered statistically significant.

Results

TGF-\( \beta \) represses miR-30 expression and HDAC inhibitor TSA blocks the repression

To verify the TGF-\( \beta \) down-regulation of miR-30s, we first treated the human podocytes with 5 ng/ml recombinant human TGF-\( \beta \) for various times and analyzed the mRNA levels of all miR-30 family members by qRT-PCR. Our results confirmed that TGF-\( \beta \) down-regulated miR-30 family expression in a time-dependent manner (Fig. 1a). To determine whether TGF-\( \beta \) repression of miR-30s is podocyte specific, we also tested miR-30d expression in HEK293 cells. We found that TGF-\( \beta \) time- and dose-dependently decreased miR-30d expression (Fig. 1b, c), which was not reported previously. Interestingly, we found that a general histone deacetylase inhibitor trichostatin A (TSA) effectively prohibited the TGF-\( \beta \)-induced down-regulation of all miR-30 family members, suggesting that histone deacetylases participate in the TGF-\( \beta \) repression of miR-30s.

TGF-\( \beta \) down-regulated miR-30d promoter transactivation

Transcriptional regulation of miR-30 has been largely unknown, mainly due to the difficult determination of their promoter locations. A recent study reported that the primary miR-30d transcript contained 4058 nucleotides and the pre-miR-30d started from +1640 of the putative transcription start site (TSS) [19]. To determine whether TGF-\( \beta \) regulates miR-30d through a transcription event, we cloned a 1.8 kb miR-30d promoter and constructed a promoter-luciferase reporter plasmid. We first tested the reporter plasmid in HEK293 cells since it supported TGF-\( \beta \) repression of miR-30s as demonstrated in Fig. 1b, c. The results showed that TGF-\( \beta \) repressed the reporter luciferase activity in a dose-dependent fashion (Fig. 2b). One previous study reported that miR-30d promoter contained a functional FOXO3\( \alpha \) binding element that was involved in miR-30d transcriptional regulation [19]. The 1.8 KB miR-30d promoter we cloned contained multiple Smad binding element-like sequences (TCTG) near the transcription starting site (TSS). To determine which part of the promoter is responsible for the TGF-\( \beta \) down-regulation and

![Graphs illustrating the down-regulation of miR-30s and miR-30d by TGF-\( \beta \) and TSA.](image-url)
whether FOXO3α is involved, we constructed a series of 5′ and 3′-truncated reporter plasmids as depicted in Fig. 2a. When the plasmids were tested in HEK293 cells, P2* and P3* that only differed by a FOXO3α binding element were not inhibited by TGF-β, while the positive control 3TP-luc plasmid was up-regulated by TGF-β as expected, indicating that FOXO3α was not involved in TGF-β down-regulation of miR-30d (Fig. 2c). On the other hand, the full length and all 5′-deletion promoter plasmids showed the similar degree of repression by TGF-β, suggesting that P4 contained the necessary components for the TGF-β repression (Fig. 2c). A computer-wised search revealed that P4 contains three putative Smad binding elements (SBEs). To confirm that the P4 conferred TGF-β repression, we performed chromatin immune-precipitation (ChIP) assay with a ChIP grade antibody that detected both Smad2 and Smad3. The results demonstrated that Smad2/3 was recruited to the −344/+14 locus, but not to the −1799/−1498 region where no SBE was predicted, upon TGF-β stimulation (Fig. 2d), suggesting that TGF-β down-regulated miR-30d through a Smad2/3-directed signaling.

**TGF-β repression of miR-30d requires histone deacetylase**

Smad signaling can cause both transcription activation and repression, in which Smad recruits histone deacetylases (HDAC) and transcription repressors to negatively regulate the downstream targets [22]. To explore the possible HDAC involvement in the repression, we first examined the effect of HDAC inhibition on the miR-30d promoter transcription activity. We treated cells transfected with miR-30d promoter reporter with a general HDAC inhibitor TSA and measured the luciferase activities. TSA alone had no effect on miR-30d promoter transcription, but when used in combination with TGF-β, it significantly reversed TGF-β-induced miR-30d promoter repression (Fig. 3a), indicating that HDAC activity was involved in Smad transcriptional repression of miR-30d. To search for the specific HDAC subtype responsible for the repression, we selectively tested a few HDACs that were related to Smad function based on previous studies [23, 24]. Notably TGF-β did not affect the protein levels of HDAC1, 2, 4, or 6, but dose-dependently enhanced HDAC3 (Fig. 3b, HDAC3-1). In another independent experiment, a less exposed blot showed more remarkable HDAC3 up-regulation by TGF-β (Fig. 3b, HDAC3-2). To further determine the role of HDAC3 in the TGF-β repression of miR-30d, we employed two strategies: the genetic HDAC3 knockdown by shRNA and a specific HDAC3 inhibitor RGFP966 prevented TGF-β-induced miR-30d promoter repression (Fig. 3d, e). We then further examined endogenous miR-30d in podocytes and found that both HDAC3
Fig. 3  TGF-β repression of miR-30d involves HDAC3. a HEK293 cells were transfected with reporter plasmid P1 and a renilla luciferase plasmid for 24 h first, then cells were treated with TGF-β (5 ng/ml) with or without TSA (30 ng/ml) for another 24 h before the luciferase activities were assessed. b Human podocytes were treated with TGF-β (0, 0.1, 1, and 5 ng/ml) for 24 h, then HDAC1, 2, 3, 4, and 6 were tested by western blotting. c Human podocytes were transiently transfected with shRNA plasmids for HDAC3 or a scrambled shRNA plasmid. HDAC3 protein level was determined by western blotting. d, e HEK293 cells were transfected with HDAC3 shRNA (d) or treated with RGFP966 (e). The cells were then treated with TGF-β (5 ng/ml) for 24 h. Luciferase assay was performed to measure the miR-30d promoter-reporter luciferase activity. f, g human podocytes were transfected with HDAC3 shRNA (f) or treated with RGFP966 (10 μM) (g). The cells were then treated with TGF-β (5 ng/ml) for 24 h. The levels of endogenous miR-30d were assayed by qRT-PCR. *p<0.05. All the figures were representative and the statistical analyses were performed with results from at least three independent experiments.

Therefore, we used a flag-tagged HDAC3 that can be detected by a stronger flag antibody M2 (Sigma, USA). We transfected HEP293 cells with the flag-HDAC3 plasmid and treated cells with TGF-β and/or TSA, then performed co-immunoprecipitation reciprocally with anti-flag, anti-Smad2/3, or anti-NCoR antibody. The results showed that TGF-β induced HDAC3 association with both Smad2/3 and NCoR, and the associations were reduced by TSA treatment (Fig. 4b, c). To characterize the role of HDAC3 in the transcription factor/DNA binding on miR-30d promoter and the effect on chromatin acetylation, we tested the effect of HDAC3 inhibition on the binding of Smad2/3, HDAC3, and NCoR to the miR-30d promoter by ChIP. Interestingly, we observed that both TSA and RGFP966 reduced the binding of Smad2/3, HDAC3, or NCoR to the miR-30d proximal promoter (−344/+14) in HEK293 and podocytes (Fig. 4d–g). Consequently, the histone 3 acetylation on the same location was increased, presumably due to the release of HDAC3 and the inhibition of its activity (Fig. 4d). These results are in line with our previous observation that HDAC3 inhibition exerts a positive role on miR-30d transcription.

**HDAC3 is critical in TGF-β-induced miR-30d down-regulation and podocyte damage**

We have previously identified Notch1 and p53 as the direct targets of miR-30 [5]. TGF-β-induced miR-30 repression caused the increased expression of Notch1 and p53, and subsequently, led to podocyte cytoskeleton rearrangement and apoptosis [5]. To investigate the effect of HDAC3 inhibition on miR-30 target
expression and the podocyte affection, we tested p53 and Notch1 protein levels in the absence or presence of TSA/RGFP966. We found that both TSA (Fig. 5a, b) and RGFP966 (Fig. 5c, d) significantly reduced the levels of Notch1 and p53 induced by TGF-β.

The effects of TSA and RGFP966 as HDAC inhibitors were verified under the same condition by western blotting, which displayed constant HDAC3 protein levels and enhanced histone 3 acetylation (Fig. 5a, c). We also examined the effect of HDAC3 inhibition on the podocyte stress fiber rearrangement, an indication of podocyte damage, by F-actin fluorescent staining. Both TSA and RGFP966 treatments markedly recovered the stress fiber loss due to TGF-β insults (Fig. 5e, f). In addition, the podocytes pre-treated with RGFP966 significantly lower the percentage of Annexin V positive apoptotic cells induced by TGF-β treatment (Fig. 5g, h). Together, these results project HDAC3 as a center player that mediates TGF-β-induced miR-30d repression and the subsequent podocyte injuries.

Discussion

In this study, we have made several novel observations: (1) miR-30d promoter is negatively regulated by TGF-β, therefore TGF-β regulation of miR-30 is a transcriptional
repression event; (2) TGF-β down-regulates miR-30 through Smad signaling pathway that involves HDAC3-associated histone deacetylation; (3) HDAC3 and NCoR are recruited by Smad2/3 to mediate miR-30d repression by TGF-β; (4) HDAC3 acts as a critical player in TGF-β-induced miR-30d repression and podocyte damage.

Recent studies reveal that miRNA promoter elements closely resemble those of protein coding regions [29], and a variety of miRNA genes are regulated by TGF-β/Smad pathway [30]. TGF-β regulates its miRNA targets mainly through Smad signaling pathway. For example, TGF-β induces both miR-216a and miR-217 in glomerularenesial cells via Smad binding elements (SBEs) in the miR-216 promoter [10]. TGF-β increases miR-143 and miR-145 through activation of myocardin and myocardin-related transcription factors (MRTFs) that associates with the promoters [31], suggesting that TGF-β/Smads can also indirectly modulate miRNA levels through other transcription factors.

In addition, TGF-β/Smad signaling also regulates miRNA by non-transcriptional mechanisms. TGF-β up-regulates miR-21 through Smad binding to CAGA sequences in the pre-miR-21 thereby promoting miR-21 maturation [32]. The regulation of miR-30s remains largely unknown. One recent study discovered that the primary miR-30d transcript is 1864 nucleotides in length and the transcription factor FOXO3α regulates its basal expression [19]. We cloned a 1.8 kb fragment of miR-30d promoter based on this study and found that miR-30d promoter contains several TGF-β-responsive elements. We further found that the FOXO3α is not involved in TGF-β down-regulation of miR-30d since neither the...
promoter P2* nor P3* show marked TGF-β repression, while the promoter P4 is sufficient to confer the TGF-β repression. These results indicate that TGF-β repression of miR-30d is promoter-dependent and the repression is a transcription regulation event. In light of the fact that miR-30b and miR-30d are encoded on one transcript and all the miR-30 family members respond similarly to TGF-β [6], we speculate that TGF-β represses the other members of miR-30 in a similar transcription repression mechanism although it warrants further investigation.

In addition to the Smad pathway, TGF-β can alternatively activate other signaling pathways, such as the extracellular signal-regulated kinase (ERK), Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinase (MAPK) pathway, the tyrosine kinase Src, and phosphatidyl inositol 3’-kinase [32], thereby affect the downstream gene expression. We performed two experiments to clarify the role of Smad2/3 signaling in the repression and found that (1) a minimal miR-30d promoter (P4) of 150 base pairs that contained three SBE-like motifs (TCTG) conferred TGF-β repression to a similar extent to that of the 1.8 kb promoter (Fig. 2c); (2) ChIP assay detected a direct association of Smad2/3 with the SBE-rich region in response to TGF-β, but not to the distant region of the same promoter (Figs. 2d, 4a, d, e). Our results suggest that miR-30d is a direct Smad2/3 target and provide the basis for the further characterization of Smad2 signaling and the miR-30 transcription regulation.

It is generally accepted that TGF-β/Smad signaling can either up- or down-regulate its targets, depending on the subcellular environment of the transcription context [33]. Numerous protein-coding genes are up-regulated by TGF-β through Smad signaling while few genes are down-regulated and the underlined molecular mechanism is poorly understood. Smads can recruit transcription co-activators such as CBP, p300, and P/CAF, which contain intrinsic histone acetyltransferase, to promote trans-activation [34]. On the other hand, a Smad repression model suggests that Smads can recruit transcription corepressors such as p107 [35], Ski, or NCoR/mSin3/HDAC [23, 36] to repress the downstream genes. We found by ChIP experiment that Smad2/3, HDAC3, and NCoR all accumulated around the miR-30d promoter upon TGF-β stimulation. The immunoprecipitation assay further confirmed that HDAC3 was physically associated with Smad2/3 and NCoR in response to TGF-β (Fig. 4b). In addition, the complex was dynamically regulated by HDAC3 since general HDAC inhibitor TSA or the specific HDAC3 inhibition reduced the complex binding to the promoter region (Fig. 4d) and led to the disassociation of HDAC3 with Smad2/3 and NCoR (Fig. 4d, e). We assume that the miR-30d repression by TGF-β might be due to HDAC3 associated histone deacetylation since HDAC3 accumulation on the miR-30d promoter induced a deacetylated histone3, which could be reversed by HDAC3 inhibition, as evidenced by ChIP assay (Fig. 4d, e, lower panel). These results support a transcription repression model in which TGF-β induces a transcription repression complex composed of at least Smad2/3, HDAC3, and NCoR on the miR-30d promoter that mediates the miR-30d repression.

Histone acetylation has been reported as a common and important epigenetic modification in kidney development and pathogenesis [37]. HDAC inhibition exerts anti-fibrotic and anti-inflammation functions in numerous animal models of kidney diseases [37, 38]; however, the particular HDAC isotypes involved in different disease models or cell types are not fully revealed. Recent studies showed that HDAC2 [39] was up-regulated by TGF-β in diabetic kidney and HDAC4 [40] was involved in STZ-induced diabetic rat model, indicating that one or several HDAC members might be actively involved in a specific disease setting. In our study, we found the HDAC3, but not other HDAC subtypes, was specifically up-regulated by TGF-β in podocytes. We further proved that HDAC3 was critical in TGF-β induced miR-30d down-regulation and podocyte injury (Fig. 5e, g). These results are consistent with previous study showing that HDAC3 is frequently associated with Smad transcription repression [41] and confirm that HDACs are selectively involved in specific kidney cell type during the disease progression.

In conclusion, we have made initial characterizations on how TGF-β represses miR-30d in podocytes. We have determined that Smad signaling directs the TGF-β down-regulation of miR-30d and HDAC3 plays a critical role. Numerous studies suggest that HDAC inhibition is renal-protective in several kidney disease models. Our results help a better understanding of podocytopathy and provide new insights into potential molecular targets for the treatment of podocyte-associated kidney diseases.

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Conflict of interest The authors declare that they have no competing interests.

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