Blocking core fucosylation of TGF-β1 receptors downregulates their functions and attenuates the epithelial-mesenchymal transition of renal tubular cells

Hongli Lin (林洪丽), 1 Dapeng Wang (王大鹏), 1 Taihua Wu (吴泰华), 2 Cui Dong (董翠), 1 Nan Shen (沈楠), 1 Yuan Sun (孙源), 1 Yanling Sun (孙艳玲), 1 Hua Xie (谢华), 1 Nan Wang (王楠), 1 and Lujuan Shan (单路娟) 2

1 Department of Nephrology and 2 Central Laboratory, The First Affiliated Hospital of Dalian Medical University, Dalian, China

Submitted 29 July 2010; accepted in final form 9 January 2011

RENAL INTERSTITIAL FIBROSIS (RIF), which results in the irreversible loss of kidney function, is a common feature of diverse kidney diseases (12, 27). However, there are currently no effective therapeutic strategies for treatment of RIF, partially due to the limited understanding of the pathophysiology and mechanism of this condition.

Burns et al. (4) confirmed that renal tubular epithelial-mesenchymal transition (EMT), which is regulated by numerous growth factors and hormones, contributes to RIF. It is generally accepted that transforming growth factor-β (TGF-β) is one of the strongest promoters of the EMT (1, 3, 6, 17). However, TGF-β is also a multifunctional factor, binds to diverse receptors, and has various physiological and pathophysiological functions through different signal pathways (1, 3). Among these signal pathways, a predominant one responsible for profibrotic function in renal tubulointerstitium is TGFβ/Smad2/3 signaling, which is initiated by activation of TGF receptors TGF-βRII and TGF-βRI (ALK5), followed by phosphorylation of Smad2/3 (10, 22, 23). Thus inactivation of TGFβ/Smad2/3 signaling has been considered a promising therapeutic approach for renal diseases treatment for nearly 20 years.

Previous studies also showed that downregulated TGF-β1 expression by treatment with a natural inhibitor, monoclonal anti-TGF-β antibody or antisense TGF-β oligonucleotides, could attenuate kidney injury (2, 9, 26, 38). However, inhibition of TGF-β1 expression has not been implemented clinically, because this approach also blocks the other vital functions of this molecule. Therefore, from a clinical viewpoint, it is important to develop a novel therapy to more specifically suppress TGF-β/Smad2/3 signaling activation. Because the variety of TGF-β functions are dependent on its receptor, suppression of TGFβ/Smad2/3 signaling activation by down-regulation of its profibrotic TGF-β receptors, ALK5 and TGF-βRII, might be a relatively effective and specific route for therapeutic intervention against EMT.

Both TGF-βRII and ALK5 are glycoproteins. As a key posttranslational modification, emerging evidence indicates that glycosylation can have profound effects on physiological processes, including cell growth, migration, and differentiation (5, 8, 19, 24, 29, 30, 33, 37), all of which closely correlate with kidney diseases. Significantly, glycosylation is now also considered a target for new drug development (25). Therefore, we hypothesized that the glycosylation of TGF-βRII and ALK5 may play a key role in EMT. If true, then blocking this process may disrupt TGF-β/Smad2/3 signaling activation and provide yet another new therapeutic avenue to disrupt EMT.

Wang et al. (34) showed that TGF-βRII was modified by core fucosylation that was dependent on Fut8, a fucosyltransferase that specifically catalyzes the introduction of fucose to position 6 of the initial N-acetyl glucosamine residue of the N-glycan core to produce “core fucose” (32, 35). Wang et al. (34) demonstrated that Fut8 knockout mice had a marked dysregulation of TGF-βRII and developed an emphysema-like phenotype in lung tissue due to deletion of core fucosylation of TGF-βRII. However, it is still unknown whether the function of TGF-βRII is dependent on its core fucosylation in renal cell EMT. Since ALK5 is another component of the “TGF-β/TGF-βRII/ALK5” complex of TGFβ/Smad2/3 signaling, whether it
is also modified by core fucose and what its possible role may be are still not clear.

Thus, in this study, we analyzed in EMT of HK-2 cells the effect of core fucosylation of two crucial TGF-β receptors, TGF-βRI (ALK5) and TGF-βRII. This is the first study on the role of TGF-β receptor fucosylation in kidney diseases.

MATERIALS AND METHODS

Reagents. Fut8 small interfering (si) RNA fragments were purchased from GenePharma (Shanghai, China). Biotinylated Lens culinaris agglutinin (LCA-Biotin), rhodamine-labeled L. culinaris agglutinin (LCA-TRITC), and fluorescein-labeled L. culinaris agglutinin (LCA-FITC) were purchased from Vector (Burlingame, CA). Anti-fibroblast-specific protein-1 (FSP-1) was purchased from LifeSpan BioSciences (Seattle, WA). Anti-Fut8 antibody, anti-Smad 2/3 antibody, anti-p-Smad 2/3 antibody, anti-TGFβRII antibody, anti-ALK5 antibody, anti-GAPDH antibody, anti-E-cadherin antibody, anti-N-cadherin antibody, horseradish peroxidase-conjugated-avidin (HRP-avidin), and Protein-G-PLUS were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-α-smooth muscle actin (SMA) antibody was purchased from Boster Biological Technology (Wuhan, Hubei, China). Lipofectamine 2000, ethidium bromide, FBS, TRIzol and DMEM were purchased from Invitrogen (Carlsbad, CA). An anti-rabbit PV kit was purchased from Santa Cruz Biotechnology (Haimen, Jiangsu, China). A SYBR PrimerScript RT-PCR Kit was purchased from Takara (Takara, Otsu, Shiga, Japan). Horseradish peroxidase (HRP)-goat anti-rabbit antibody, HRP-goat anti-mouse antibody, HRP-rabbit anti-goat antibody, and FITC-goat anti-rabbit antibody and FITC-rabbit anti-goat antibody were purchased from Beijing Zhong Shan-Golden Bridge Biological Technology (Beijing, China). An enhanced chemiluminescence (ECL) detection system was purchased from Amersham Biosciences (Pittsburgh, PA), and a BCA protein assay kit from Pierce (Rockford, IL).

Cell culture. Immortalized human renal proximal tubular epithelial (HK-2) cells were purchased from American Type Culture Collection (Manassas, VA). They were cultured in DMEM medium supplemented with 10% FBS and plated at a density of 2 × 105 cells/well in six-well plates (Costar, Corning). The cells were randomly divided into six groups: 1) normal control: cells were cultured in DMEM medium; 2) mock group: transient transfection with 30 nM scrambled siRNA for 72 h; 3) TGF group: cells were treated with TGF-β1 at 5 ng/ml for 48 h to construct an EMT model in HK-2 cells; 4) TGGF group: transfection with 30 nM Fut8siRNA for 24 h and then with 5 ng/ml TGF-β1 for 48 h; 5) TGFM group: transfection with 30 nM Fut8siRNA for 24 h and then with 5 ng/ml TGF-β1 for 48 h; and 6) Fut8 siRNA group: transfection with 30 nM Fut8siRNA for 72 h.

siRNA design, preparation, and transfection. Chemically synthesized Fut8-siRNAs were custom designed to target the Fut8 gene. The siRNA sequences were identified using BLAST and the human genome database to assess possible cross-reactivity. Four siRNAs (5′-UCCGACACCGAUCACGACA-3′, 5′-GGGUUCUCUAGGU-UCGAA-3′, 5′-GGUCAGUUGAAGAAACATTT-3′, and 5′-GGUG-UCUAAUCACAAATT-3′) were synthesized and pooled, and the dried siRNA pools were reconstituted in DEPC-treated H2O to a final concentration of 30 nM and stored at −20°C until use. For cell transfection, cells were added to six-well culture plates and incubated for 24 h to allow cell multiplication in an antibiotic-free medium. Then, the siRNAs and transfection reagent were complexed as recommended by the manufacturer and added to the cell culture wells.

Real-time RT-PCR analysis. Total RNA was extracted from the HK-2 cells using TRIzol according to the manufacturer’s instructions. The first-strand cDNA synthesis was carried out using a SYBR PrimerScript RT-PCR Kit according to the manufacturer’s instructions. Relative levels of mRNA for Fut8 were determined by real-time PCR with a LightCycler (Roche, Mannheim, Germany) according to the manufacturer’s manual. The following two sets of primers, 5′-TGACTCGTCAATCTGACGAGGA-3′ (forward) and 5′-AGTTT- GCAGAGGCATCAGGATGTAG-3′ (reverse), were for Fut8; and 5′-GAAGGTGAAGGTCGGAGT-3′ (forward) and 5′-GAAGTAGT-GGTAGGGGATTTC-3′ (reverse) were for GAPDH. PCR products obtained after 30 s at 95°C, followed by 40 cycles of 5 s at 95°C, 20 s at 60°C, and 30 s at 72°C, were assessed for specificity by melting curve analysis. To control for variations in the amount of DNA available for PCR in the different samples, the levels of gene expression of the target sequence were normalized in relation to that of the housekeeping gene GAPDH. All samples were analyzed in triplicate.

Fucosylation detection. LCA-FITC was used to detect the expression of core fucose in HK-2 cells. Cells were fixed (12,000 rpm, 20 min, 4°C), and the supernatant was collected and precleared by Protein G PLUS-Agarose, after which the whole cell lysates (500 μg) were incubated with 2 μg of anti-TGFβRII or anti-ALK5 at 4°C for 2 h on a rocker platform (30 rocks/min), respectively. Protein-antibody complexes were then collected with 20 μl of Protein G PLUS-Agarose at 4°C on a rocker platform (30 rocks/min) overnight. For negative control, the primary antibody was omitted. The immunoprecipitate was washed three times with lysis buffer. Equal amounts of proteins were subjected to 12% SDS-PAGE for lectin blotting (described below).

Lectin blotting. Immunoprecipitated TGFβRII or ALK5 was separated by 12% SDS-PAGE and electroblotted onto polyvinylidene difluoride (PVDF) membranes (Bio-Rad). The membranes were blocked with 5% BSA (wt/vol) in Tris-buffered saline containing 0.1% Tween 20 (TBST) at 4°C overnight, and then incubated for 1 h at 23°C in TBST containing LCA-Biotin (1:200), which preferentially recognizes Fuc-1,6GlcNAc. After a wash with TBST four times, lectin-reactive proteins were detected using an ECL kit.

Western blotting. Cell cultures were harvested and dissolved in RIPA buffer, lysates were centrifuged at 15,000 g for 10 min at 4°C, and supernatants were then collected. Protein concentrations were determined using a BCA protein assay kit. Protein samples were then denatured at 100°C for 3 min, separated by 12% SDS-PAGE, and electroblotted onto PVDF membranes (Bio-Rad). Blots were probed with appropriate primary antibodies at 4°C overnight to detect TGF-βRII, ALK5, Fut8, Smad 2/3, p-Smad 2/3, E-cadherin, α-SMA, N-cadherin expression. Next, the blots were incubated with HRP-labeled secondary antibody for 1 h at 23°C, followed by detection with ECL. Band intensity was quantified using LabWorks Image Acquisition and Analysis software.

Double-immunostaining of core fucose and TGF-βRII and core fucose and ALK5. Cells were applied with rabbit anti-TGF-βRII antibody (1:500) or rabbit anti-ALK5 antibody (1:500) and LCA-TRITC (1:1,000), followed by the incubation of FITC-goat anti-rabbit antibody (1:200) for 40 min at 23°C away from the light. After washing of the cells in 0.1 M PBS, the expressions of LCA and TGF-βRII and LCA and ALK5 were observed with fluorescence microscopy.

Immunocytochemical and immunofluorescent analysis. For immunoocytochemistry of E-cadherin, FSP-1, and N-cadherin, HK-2 cells were fixed for 30 min on coverslips using 0.01 M PBS containing 4% paraformaldehyde, and then blocked for 15 min with 0.3% H2O2.
After incubation with 3% goat serum or rabbit serum for 30 min at 23°C, cells were incubated with anti-E-cadherin antibody, anti-FSP-1 antibody, or anti-N-cadherin antibody at 4°C overnight; HRP-goat anti-rabbit antibody was then added for 60 min at 23°C. The reaction products were visualized with an anti-rabbit PV kit, according to the manufacturer’s instructions.

For p-Smad2/3 immunofluorescence, HK-2 cells were incubated at 4°C overnight with goat anti-p-Smad2/3 antibody (1:150), followed by incubation of FITC-rabbit anti-goat antibody (1:100) for 1 h at 23°C. After washing in 0.01 M PBS, the reaction was observed by fluorescence microscopy.

**Statistical analysis.** All values are expressed as means ± SD. Statistical analysis was performed by ANOVA with post hoc analysis using Tukey’s test, and a P value <0.05 was considered to indicate a statistically significant difference.

**RESULTS**

**Core fucosylation is upregulated during renal tubular EMT.** Changes in core fucosylation are associated with some pathological processes (30), but the core fucosylation has not yet been studied in HK-2 cells. Treating HK-2 cells with TGF-β1 could mimic the EMT process in vitro, so we initially successfully constructed this in vitro model by treating HK-2 cells with 5 ng/ml human recombinant TGF-β1 for 48 h and investigated whether the core fucosylation changed during the process of EMT by using fluorescein-labeled LCA-FITC. Our data showed that the LCA-FITC staining was obviously enhanced in cells after EMT of HK-2 cells (Fig. 1).

**Establishment of Fut8-knockdown in HK-2 cells.** To address the role of core fucosylation in EMT of HK-2 cells, we sought to downregulate core fucosylation in HK-2 cells. Toward this end, we used RNA interference to block the expression of Fut8 in HK-2 cells. Four Fut8-siRNAs were designed and chemically synthesized as a Fut8 siRNA pool targeting the Fut8 gene and transfected into HK-2 cells using Lipofectamine 2000. As shown in Fig. 2, the expression of exogenous Fut8 siRNA in HK-2 cells was detectable at 12 h after transfection (Fig. 2B), reached its peak value at 48 h (Fig. 2D), and remained elevated at 72 h (Fig. 2E). The results of real-time RT-PCR (Fig. 3A) and Western blotting (Fig. 3B) confirmed that the Fut8-siRNAs

---

Fig. 1. Core fucose is present in HK-2 cells. A: core fucose existed on the surface and in the cytoplasm of HK-2 cells of the normal group, as indicated by *Lens culinaris* agglutinin (LCA)-FITC staining. B: core fucose increased in cells of the transforming growth factor (TGF) group. C: negative control. Original magnification ×100.

Fig. 2. Time course of Fut8 small interfering (si) RNA transfection of HK-2 cells. HK-2 cells were transfected with fluorescently labeled Fut8 siRNA (30 nM). Representative photomicrographs show exogenous Fut8 siRNA expression and localization in HK-2 cells before (0 h; A) and at 12 (B), 24 (C), 48 (D), and 72 h (E) after transfection. Original magnification ×400. Each experiment was performed in triplicate.
effectively decreased the Fut8 mRNA and protein expression in HK-2 cells in both the TGFF and Fut8 siRNA groups.

**Fut8 siRNA decreased core fucosylation in the EMT of HK-2 cells.** We previously demonstrated that core fucosylation is upregulated in EMT (Fig. 1) and also artificially decreased the expression of the key fucosyltransferase that is involved, Fut8 (Fig. 3). Therefore, we further investigated whether Fut8 siRNA could decrease core fucosylation in the EMT of HK-2 cells. As shown in Fig. 3C, 30 nM Fut8 siRNA effectively deleted core fucosylation of normal HK-2 cells (presented in the Fut8 siRNA group) and reversed the increasing of core fucosylation in cells of the TGFF group compared with the TGF group.

**TGF-βRII and ALK5 are target proteins modified by core fucosylation.** Knowing that core fucosylation of HK-2 cells is increased in EMT, as demonstrated in the previous section, it was important to determine which proteins were involved. Since TGF-βRII and ALK5 are two important receptors of the TGF-β/Smad2/3 pathway, we examined whether these two key glycoproteins were modified by core fucosylation in HK-2 cells. Figure 4, A–D, shows that the positive bands of core fucose were present in TGF-βRII and ALK5 immune precipitates, which indicates that TGF-βRII and ALK5 were indeed modified by core fucosylation. Next, the spatial relationship of core fucose with TGF-βRII and ALK5 was examined by immunofluorescence double-staining. The expression epitopes of core fucose completely overlapped with both TGF-βRII and ALK5 in HK-2 cells, further confirming that both TGF-βRII and ALK5 are modified by core fucosylation in HK-2 cells (Fig. 4, E and F).

**Fut8 siRNA suppresses TGF-βRII and ALK5 core fucosylation, but not their protein overexpression in EMT.** We also found that the protein expression of TGF-βRII and ALK5 increased in parallel with their posttranslational core fucosylation upregulation with EMT of HK-2 cells (Fig. 4, A–D, TGF group). The enhanced core fucosylation of TGF-βRII and ALK5 was significantly inhibited by Fut8 siRNA in the TGFF group compared with the TGF group, while the overexpression of TGF-βRII and ALK5 were not affected (Fig. 4, A–D). The immunofluorescence double-staining also indicated that the core fucosylation of TGF-βRII and ALK5 were significantly elevated in the TGFF group compared with the normal and mock groups, but obviously reduced in Fut8 siRNA transfectants (Fig. 4, E and F).
Abolishing TGF-βRII and ALK5 core fucosylation by Fut8 siRNA inhibits Smad2/3 phosphorylation and nuclear translocation. Since posttranslational modifications may affect protein function, we further studied whether blocking core fucosylation of ALK5 and TGF-βRII with Fut8 siRNA could suppress the activation of the TGF-β/Smad2/3 pathway, by investigating its effects on the phosphorylation and nuclear translocation of Smad2/3. Western blotting results showed that the increased expression
of p-Smad2/3 after TGF-β1 stimulation was significantly decreased in the TGFF group compared with the TGF group, due to downregulation of core fucosylation of ALK5 and TGF-βRII (Fig. 5, A and B). Furthermore, we found that p-Smad2/3 was distributed strictly in the cytoplasm of normal HK-2 cells by immunofluorescence staining (Fig. 5C). After the cells were treated with TGF-β1, the intensity of p-Smad2/3 was significantly upregulated and the immunostaining was translocated into the nuclei of HK-2 cells in the TGF group. Eliminating core fucosylation effectively inhibited the overexpression and nuclear translocation of p-Smad2/3 in HK-2 cells (Fig. 5C).

**Blocking core fucosylation has protective effects on EMT.** Blocking core fucosylation by silencing Fut8 expression inhibited the activation of the TGF-β/Smad2/3 pathway in HK-2 cells, which led us to further investigate whether Fut8 siRNA could attenuated the phenotypic changes of HK-2 cells during the EMT. The phase-contrast microscopy results indicated that normal HK-2 cells exhibited typical cobblestone morphology with an epithelial phenotype when cultured in DMEM. After incubation with TGF-β1 for 48 h, the cells elongated, developed a spindle shape, disassociated from neighboring cells, and lost their cobblestone monolayer arrangement and became disordered. Pretransfection of cells with Fut8 siRNA for 24 h attenuated these phenotypic changes, and most had normal epithelial cell morphology (Fig. 6).

Many studies have demonstrated that renal tubular epithelial cells change into myofibroblasts characterized by reduced expression of E-cadherin and increased expression of α-SMA, N-cadherin, and FSP-1. Therefore, we examined the expression of α-SMA, E-cadherin, N-cadherin, and FSP-1 by Western blotting and immunocytochemistry (Fig. 7). When HK-2 cells were incubated for 48 h with 5 ng/ml TGF-β1, the expression of α-SMA, N-cadherin, and FSP-1 was significantly increased, while that of E-cadherin was markedly decreased. Pretransfection of the cells of the TGFF group with Fut8 siRNA for 24 h clearly attenuated these changes (Fig. 7).

**DISCUSSION**

In some pathological processes, the increased expression of a protein may not reflect its pathological function due to its posttranslational modification (21) and may even present conflicting phenomena (11). Therefore, research focused only on the change in the expression of a target protein in a particular pathologic state is not necessarily enough to adequately analyze the situation. As one of the most important of posttranslational modifications, glycosylation can regulate protein functions and has profound effects on physiological or pathological processes such as cell growth, migration, and differentiation, and has therefore been increasingly explored in new biomarker and drug development (7, 13–16, 18, 28, 31, 36). Heretofore, there has been no study focusing on the role of N-glycosylation of proteins involved in kidney diseases, and this is the first report concerning core fucosylation in renal cells. In the present study, we constructed a typical model of EMT of cultured HK-2 cells with TGF-β1 in vitro, which was confirmed by biomarker and morphological evidence (TGF group: Figs. 6 and 7), and used the model to investigate the role of core fucosylation in EMT.

It was unclear whether core fucose exists in HK-2 cells, so we first confirmed by immunofluorescence that it indeed existed in these cells. The level of core fucose was investigated and found to be increased in cells of the EMT (Fig. 1). These results suggest that core fucosylation is upregulated in EMT of
HK-2 cells and may play a pathological role in this process. Therefore, to further address this role, we sought to delete core fucosylation in HK-2 cells. Since the core fucosylation depends on Fut8, a fucosyltransferase that catalyzes the fucosylation of N-glycans to produce core fucose, we designed and synthesized a Fut8 siRNA pool and transfected it into HK-2 cells (Fig. 2). The results showed that synthetic Fut8 siRNAs successfully silenced Fut8 expression at both the mRNA and protein levels (Fig. 3, A and B) and eliminated core fucosylation in HK-2 cells (Fig. 3C).

As TGF-βRII and ALK5 are two key initial receptors of TGF-β signaling, we investigated whether they were modified by core fucosylation using Western and lectin blotting. In a previous study of Fut8-null mice, Wang et al. (34) demonstrated that Fut8 catalyzed the core fucosylation of TGF-βRII. However, in our study, we found that not only TGF-βRII but also ALK5 was modified by core fucosylation catalyzed by Fut8 (Fig. 4). We also found that TGF-β1 significantly increased the expression of TGF-βRII and ALK5 protein in HK-2 cells (Fig. 4, A–D), in agreement with several previous studies (10, 20). Interestingly, we found that Fut8 siRNA disrupted fucosylation of TGF-βRII and ALK5, while it did not affect their protein expression (Fig. 4, A–D). In particular, the expression epitopes of core fucose and TGF-βRII (Fig. 4E), core fucose and ALK5 (Fig. 4F) overlapped completely, which agreed with the results of Western and lectin blotting. These results suggest that the effects of fucosylation of TGF-βRII and ALK5 on EMT are independent of TGF-βRII and ALK5 protein expression.

Smad2/3 phosphorylation and nuclear translocation are markers of TGF-β/Smad2/3 signaling activation. To determine whether core fucosylation can regulate activation of such signaling, Smad2/3 phosphorylation and nuclear translocation were studied. In support of this hypothesis, inhibition of core fucosylation of TGF-βRII and ALK5 was found to block Smad2/3 phosphorylation (TGFF group: Fig. 5, A and B) and nuclear translocation (TGFF group: Fig. 5C) in HK-2 cells. To gain direct evidence of the effects of core fucosylation on EMT, the effects of loss of core fucosylation of TGF-βRII and ALK5 on EMT were also assessed by investigating changes in the appearance of HK-2 cells and EMT-associated protein markers. The results showed that depleting core fucosylation by Fut8 siRNA significantly reversed the upregulation of α-SMA (Fig. 7, A and B), N-cadherin and FSP-1 (Fig. 7, E–G), and downregulation of E-cadherin (Fig. 7, C–D and G) in EMT, and also prevented fibrotic changes in HK-2 cells (Fig. 6).

Taken together, our results indicated that Fut8 siRNA successfully inhibited the core fucosylation of TGF-βRII and ALK5 and caused loss of TGF-β/Smad2/3 signaling activation and subsequently blocked EMT development. Since it is very difficult to specifically block the core fucosylation of TGF-βRII and ALK5, we cannot exclude the possibility that some other targets of Fut8 fucosylation might be involved in EMT of HK-2 cell. However, TGF-β is the strongest profibrotic factor in EMT, and dysregulation of TGF-β may provide the most effective inhibitor in EMT. Therefore, we aimed TGF-β sig-

![Fig. 6. Fut8 siRNA inhibits phenotypic changes in HK-2 cells that occurred in the epithelial-mesenchymal transition (EMT). Representative photomicrographs indicate that normal HK-2 cells exhibited typical cobblestone morphology with epithelial cells. After incubation with TGF-β1 for 48 h, the cells elongated, developed a spindle shape, dissociated from neighboring cells, and lost their cobblestone monolayer with disordered arrangement. Fut8 knockdown significantly inhibited this effect. Phase contrast microscopy, original magnification ×100. Each experiment was performed in triplicate.](http://ajprenal.physiology.org/)

**AJP-Renal Physiol • VOL 300 • APRIL 2011 • www.ajprenal.org**
naling to investigate the effects of core fucosylation of TGF-β receptors in EMT of HK-2 cells. In summary, the current study demonstrated that blocking core fucosylation of TGF-βRII and ALK5 (Fig. 4) effectively inhibited the profibrotic function of TGF-β1, which was strongly proven by decreasing the phosphorylation and nuclear translocation of its specific downstream protein-Smad2/3 (Fig. 5). This indicates that the core fucosylation of TGF-β receptors is essential and crucial for EMT of renal cells and suggests that regulation of core fucosylation of the TGF-β receptors may be a potential therapeutic target for the treatment of renal diseases.

Fig. 7. Effects of Fut8 siRNA on the expression of α-smooth muscle actin (SMA), E-cadherin, N-cadherin, and fibroblast-specific protein-1 (FSP-1) in HK-2 cells. Western blotting indicated that E-cadherin decreased, while α-SMA and N-cadherin increased in cells of the TGF group, all of which were reversed by Fut8 siRNA transfection. Western blots and quantification of results of total cell lysates (all relative to GAPDH) for α-SMA (A and B), E-cadherin (C and D), and N-cadherin (E and F) are shown. The expression of measured protein in each sample was normalized to GAPDH expression. Values are means ± SD. *P < 0.05 vs. normal group. #P < 0.05, each group (except for the normal group) vs. TGF group. Immunocytochemistry results (G) for E-cadherin and N-cadherin were consistent with the Western blot results. Meanwhile, the expression level of FSP-1 increased in the cells of the TGF group while it was reversed by Fut8 siRNA transfection. Original magnification ×100. Each experiment was performed in triplicate.
REFERENCES


